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August 14, 2000

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Washington, DC 20231

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Presented for filing is a new patent application claiming priority from provisional patent applications of:

Applicant: PARSA KAZEMI-ESFARJANI and SEYMOUR BENZER

Title: AN ANIMAL MODEL OF POLYGLUTAMINE TOXICITY,
METHODS OF USE, AND MODULATORS OF POLYGLUTAMINE
TOXICITY

Enclosed are the following papers, including those required to receive a filing date under 37 CFR 1.53(b):

BOSTON
DALLAS
DELAWARE
NEW YORK
SAN DIEGO
SILICON VALLEY
TWIN CITIES
WASHINGTON, DC

	<u>Pages</u>
Specification	85
Claims	9
Abstract	1
Drawing(s)	68

Enclosures:

- Small Entity Statement
- Combined Declaration and Power of Attorney
- Recordation Form Coversheet, Assignment, and check for \$40 recording fee
- Postcard.

Under 35 USC §119(e)(1), this application claims the benefit of prior U.S. provisional application nos. 60/148,934, filed August 12, 1999; 60/148,933, filed August 12, 1999; 60/177,047, filed January 18, 2000; and 60/205,720, filed May 19, 2000.

Express Mail Label No EL584780899US

I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

August 14, 2000

Date of Deposit

Derek Norwood

FISH & RICHARDSON P.C.

Commissioner for Patents
August 14, 2000
Page 2

This application contains 79 total claims, 15 of which are independent. This application is entitled to small entity status.

Basic filing fee	\$345
Total claims in excess of 20 times \$9	\$531
Independent claims in excess of 3 times \$39	<u>\$468</u>
Total filing fee:	\$1,344

A check for the filing fee noted above is submitted herewith. Please apply any other fees, or apply any credits, to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at (858) 678-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

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Respectfully submitted,



43,488

Scott C. Harris *FOX*
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Enclosures

Applicant or Patentee: Parsha Kazemi-Befarjari et al.
 Serial or Patent No.:
 Filed or Issued: August 14, 2000
 For: AN ANIMAL MODEL OF POLYGLUTAMINE TOXICITY, METHODS OF USE, AND MODULATORS OF POLYGLUTAMINE TOXICITY

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(d)) — NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: California Institute of Technology
 Address of Organization: 1200 East California Blvd., Pasadena, CA 91125

Type of Organization:

- University or Other Institution of Higher Education
- Tax Exempt Under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))
- Nonprofit Scientific or Educational Under Statute of State of the United States of America
(Name of State: _____) (Citation of Statute: _____)
- Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the United States of America
- Would qualify as nonprofit scientific or educational under Statute of State of the United States of America if located in the United States of America
(Name of State: _____) (Citation of Statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled **AN ANIMAL MODEL OF POLYGLUTAMINE TOXICITY, METHODS OF USE, AND MODULATORS OF POLYGLUTAMINE TOXICITY** by inventor(s) **PARSA KAZEMI-ESFAJANI AND SEYMOUR BENZER** described in:

- the specification filed herewith.
- application serial no. _____ filed August 14, 2000.
- parent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(e) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Full Name: _____

Address: _____

INDIVIDUAL SMALL BUSINESS CONCERN NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status when any new rule 53 application is filed or prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name:	Adam Cochran
Title:	Intellectual Property Counsel
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Signature: Adam Cochran Date: 8/14/00

**APPLICATION FOR
UNITED STATES PATENT**

in the name of

**SEYMOUR BENZER
and
PARSA KAZEMI-ESFARJANI**

for

**AN ANIMAL MODEL OF
POLYGLUTAMINE TOXICITY,
METHODS OF USE, AND
MODULATORS OF POLYGLUTAMINE
TOXICITY**

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ATTORNEY DOCKET: 06618-686001
Client Ref. No.: CIT-3056

Date of Deposit: August 14, 2000
EXPRESS MAIL NO.: EL584780899US

AN ANIMAL MODEL OF POLYGLUTAMINE TOXICITY, METHODS OF USE, AND MODULATORS OF POLYGLUTAMINE TOXICITY

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to provisional application serial Nos. 60/148,934, filed August 12, 1999; 60/148,933, filed August 12, 1999; 60/177,047, filed January 18, 2000; and 60/205,720, filed May 19, 2000.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

10 This invention was made with Government support under Grant Nos. AG12289, awarded by the National Institutes of Health, and MCB-9408718, awarded by the National Science Foundation. The Government has certain rights in this invention.

TECHNICAL FIELD

15 This invention relates to an animal model that exhibits polyglutamine toxicity, and more particularly to methods for identifying genes that modulate polyglutamine toxicity using *Drosophila*.

BACKGROUND

20 Expansion of polyCAG tracts is associated with human hereditary neurodegenerative disorders and neuronal toxicity (Kaytor *et al.*, *J. Biol. Chem.*, **274**:37507-37510 (1999)). Huntington's disease and several other hereditary neurodegenerative disorders are characterized by expansion of a polyglutamine sequence (LaSpada *et al.*, *Nature*, **352**:77-79 (1991); Koide *et al.*, *Nat. Genet.*, **6**:9-13 (1994); Kawaguchi *et al.*, *Nat. Genet.*, **8**:221-228 (1994); Orr *et al.*, *Nat. Genet.*, **4**:221-226 (1993); Sanpei *et al.*, *Nat. Genet.*, **14**:277-284 (1996); and Zhuchenko *et al.*, *Nat. Genet.*, **15**:62-69 (1997)). The expanded polyCAG tracts encode abnormally long polyglutamine sequences within specific proteins promoting their nuclear and/or cytoplasmic aggregation. The protein aggregation is believed to contribute to cellular toxicity including cell death or apoptosis (Trottier *et al.*, *Nature*, **378**:403-406

(1995); Davies *et al.*, *Cell*, **90**:537-548 (1997); and DiFiglia *et al.*, *Science*, **277**:1990-1993 (1997).

The mechanism of toxicity and cell death by expanded polyglutamines is not yet fully understood. Peptides containing expanded polyglutamine tracts are prone to forming cytoplasmic (CIs) and/or nuclear inclusions (NIs). Two variables appear as major determinants of the aggregation propensity, subcellular localization or toxicity of polyglutamine-containing peptides. The relative length of the polyglutamine tract determines the aggregation propensity and cytotoxicity; the longer it is, the more likely it is to form inclusions and cause cell death. The overall size of the peptide determines subcellular localization as well as aggregation propensity and cytotoxicity; shorter, truncated gene products with expanded repeats are more likely to form inclusions, and these inclusions are more likely to be in the nucleus than in the cytoplasm. These inclusions occasionally recruit their full-length counterpart.

Perinuclear inclusions produced by truncated huntingtin peptides recruit endogenous huntingtin in transfected human kidney epithelial 293T cells (HEK 293T). Cotransfection of truncated ataxin-3 (SCA3 gene product) with its full-length counterpart, containing either a normal or an expanded polyglutamine tract, resulted in the recruitment of either of the two full-length proteins into perinuclear inclusions formed by the truncated ataxin-3. However, this type of recruitment was not observed in HD brains. In another set of experiments, huntingtin was recruited to neuritic plaques, neurofibrillary tangles and dystrophic neurites in Alzheimer's disease, and to Pick bodies found in Pick disease. Heteromeric aggregates were also formed between co-expressed ataxin-1, with normal or expanded polyglutamine, and ataxin-3 with an expanded polyglutamine repeat in transfected HEK 293T.

Experiments in mouse striatal cell culture and transgenic mice suggested that nuclear localization was necessary for the pathogenic effects. On the other hand, experiments in a human embryonic kidney cell line suggested that polyglutamine can be equally cytotoxic in the cytoplasm or the nucleus. Furthermore, in cultured mouse clonal striatal cells or in SCA1 transgenic mice, aggregation of polyglutamines appeared to be neither sufficient nor necessary for pathogenesis. When NI formation was suppressed in neurons transfected with mutant huntingtin, cell death increased.

The molecular components of the pathways involved in neuronal degeneration and protein aggregation have been investigated. These include: components of protein folding (Cummings *et al.*, *Nat Genet*, **19**:148-154 (1998); Wyttenbach *et al.*, *Proc. Natl. Acad. Sci. USA*, **97**:2898-2903 (2000); and Kobayashi *et al.*, *J. Biol. Chem.*, **275**:8772-8778 (2000)),
5 protein degradation (Chai *et al.*, *Hum. Mol. Genet.*, **8**:673-682 (1999)), gene expression (Boutell *et al.*, *Hum. Mol. Genet.*, **8**:1647-1655 (1999); Kazantsev *et al.*, *Proc. Natl. Acad. Sci. USA*, **96**:11404-11409 (1999); and Li *et al.*, *J. Neurosci.*, **19**:5159-5172 (1999)), and programmed cell death (Portera *et al.*, *J. Neurosci.*, 3775-3787 (1995); Wellington *et al.*, *J. Biol. Chem.*, **273**:9158-9167 (1998); and Ona *et al.*, *Nature*, **399**:263-267 (1999)), as well
10 as interacting proteins (Kalchman *et al.*, *Nat. Genet*, **16**:44-53 (1997); Sittler. *et al.*, *Mol. Cell*, **2**:427-436 (1998); Waragai *et al.*, *Hum. Mol. Genet.*, **8**:977-987 (1999)), neurotransmitters, and their receptors (Cha *et al.*, *Proc. Natl. Acad. Sci. USA*, **95**:6480-6485 (1998); Chen *et al.*, *J. Neurosci.*, **72**:1890-1898 (1999); and Reynolds *et al.*, *J. Neurochem.*, **72**:1773-1776 (1999)). A *Drosophila* model has recapitulated abnormal protein aggregation
15 and neuronal toxicity associated with polyglutamine disorders, and a candidate heat shock gene has been shown to have a suppressing effect (Warrick *et al.*, *Cell*, **93**:939-949 (1998); Jackson *et al.*, *Neuron*, **21**:633-642 (1998); Marsh *et al.*, *Hum. Mol. Genet.*, **9**:13-25 (2000); and Kazemi-Esfarjani, *Science*, **287**:1837-1840 (2000)). The present invention is based upon an alternative animal model that mimics polyglutamine and/or protein folding abnormalities
20 observed in humans.

SUMMARY

The present invention relates to an animal model useful for identifying molecules that modulate expression or activity of proteins involved in polyglutamine toxicity, neuronal and other degenerative disorders, cancer and other proliferative disorders in humans. This animal model is also useful for identifying molecules that modulate disorders associated with undesirable or aberrant protein folding, aggregation, degradation or aberrant transport. Such molecules include genes and other compounds that modulate protein aggregation or folding and associated disorders, including polyglutamine toxicity and polyglutamine related
25 disorders.
30

A genetic screen using a *Drosophila* animal model of the invention identified *in vivo* genetic modulators of polyglutamine toxicity. Three *Drosophila* genes, heat shock protein 40/HDJ1 (dHDJ1), tetratricopeptide repeat protein 2 (dTTPR2) and myeloid leukemia factor 1 (dMLF), were capable of decreasing polyglutamine toxicity in affected flies. Thus, the

5 *Drosophila* genes or their mammalian homologues and other compounds identified using an *in vivo* animal model of the invention can be used as therapeutics in treating polyglutamine toxicity and associated disorders in humans. A method of the invention, and the genes and compounds identified, are also applicable for the identification and treatment of disorders associated with other diseases that result from or are associated with intracellular or

10 extracellular protein misfolding/aggregation. Particular examples include Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD), Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma. In addition, as the human homologues of dTTPR2 and dMLF

15 (TPR2 and MLF, respectively) are associated with tumorigenesis (neurofibromatosis 1) and leukemias (myelodysplastic syndrome and acute myeloid leukemias), respectively, these genes, and the flies carrying dTTPR2 and dMLF P-element insertions or their transgenic versions, will be helpful in identifying cancer therapeutics.

In accordance with the present invention, there are provided methods of screening for

20 genes or compounds that modulate polyglutamine toxicity. In one embodiment, a method of the invention includes providing a first animal expressing a polyglutamine sequence, wherein the sequence produces polyglutamine toxicity in the animal; breeding the first animal to a second animal, wherein the second animal has a marker sequence inserted into its germline, thereby producing progeny; screening the progeny for increased or decreased polyglutamine

25 toxicity relative to the first animal thereby identifying a progeny having increased or decreased polyglutamine toxicity; and identifying one or more genes adjacent to or having an insertion of the marker sequence that confers increased or decreased polyglutamine toxicity in the progeny having increased or decreased polyglutamine toxicity. In another embodiment, a method further includes identifying a mammalian homologue (e.g., human

30 homologue) of the gene.

Methods of screening that are included employ first and second animal invertebrates. In one embodiment, a method includes invertebrates of the genus *Drosophila* (e.g., *Drosophila melanogaster*).

In one embodiment, a marker used in the methods and animals of the invention 5 includes a P element sequence. In another embodiment, the marker sequence comprises a polynucleotide sequence that disrupts or alters expression of one or more genes near the sequence. In yet another embodiment, a marker sequence includes an expression control element conferring expression of the one or more genes near the marker. In one aspect, the expression control element increases expression. In another aspect, the expression control 10 element decreases expression.

Methods of the invention include screening methods in which a plurality of second animals having markers located at different positions within their genome are screened. Thus, in one embodiment, a second animal is selected from a group of two or more animals having markers inserted into different locations of its genomic DNA. In another 15 embodiment, the second animal is selected from a group of 10 to 100, 100 to 500, or 500 or more of the animals. In yet another embodiment, the second animal is selected from a library of animals having markers inserted at random locations of their genomic DNA. In still another embodiment, each of the second animals is generated by random P-element insertions into the genome. In one aspect, a library of animals is generated by random P 20 element insertion.

Polyglutamine sequences of the methods and transgenic animals of the invention include, for example, sequences having between about 35 to 50 glutamine residues, between about 50 to 100 glutamine residues, between about 100 to 150 glutamine residues and having about 150 or more glutamine residues. The sequences can be encoded by a plurality of 25 CAGs, CAAs or a combination thereof. Expression of the plurality of CAGs, CAAs or combination thereof can be conferred by a constitutive, regulatable or tissue specific expression control element. In one embodiment, the regulatable element comprises an inducible or repressible element. In another embodiment, the regulatable element comprises a GAL4 responsive sequence. In yet another embodiment, the tissue specific element confers 30 neural, retinal, muscle or mesoderm cell expression.

Polyglutamine sequences can additionally include other molecular entities. In one embodiment, a polyglutamine sequence further includes a tag. In one aspect, a tag comprises an epitope tag. In another aspect, a tag comprises a hemagglutinin sequence.

Animals of the invention include progeny animals produced by the screening methods 5 of the invention that employ animals. In one embodiment, a progeny animal exhibits decreased polyglutamine toxicity relative to a parent that exhibits polyglutamine toxicity. In another embodiment, a progeny animal exhibits increased polyglutamine toxicity relative to a parent that exhibits polyglutamine toxicity.

Animals of the invention further include transgenic animals including a transgene 10 containing a plurality of CAGs and at least one CAA sequence encoding a polyglutamine repeat sequence. In one embodiment, a transgenic animal is an invertebrate. In another embodiment, a transgenic animal is of the genus *Drosophila* (e.g., *Drosophila melanogaster*).

Transgenic animals of the invention including a transgene containing a plurality of 15 CAGs and at least one CAA sequence encoding a polyglutamine repeat sequence can have any number of CAGs and CAAs in any ratio encoding the repeat sequence. In one embodiment, the number of CAGs to CAAs is in ratio of between about 1:1 and 2:1. In another embodiment, the number of CAGs to CAAs is in ratio of between about 2:1 and 5:1. In yet another embodiment, the number of CAGs to CAAs is in ratio of between about 5:1 and 10:1. In still another embodiment, the number of CAGs to CAAs is in ratio of between 20 about 10:1 and 50:1.

Thus, a transgenic animal of the invention including a transgene containing a plurality 25 of CAGs and at least one CAA sequence encoding a polyglutamine repeat sequence can express a polyglutamine repeat sequence of any length. In one embodiment, the polyglutamine sequence is between about 5 and 20 amino acids in length. In another embodiment, the polyglutamine sequence is between about 20 and 50 amino acids in length. In yet another embodiment, the polyglutamine sequence is between about 50 and 100 amino acids in length. In additional embodiments, the polyglutamine sequence is between about 100 and 200 amino acids in length, between about 100 and 500 amino acids in length and between about 50 and 200 amino acids in length. In various aspects, a polyglutamine 30 sequence further includes a tag (e.g., epitope, hemagglutinin, etc.)

In other embodiments, expression of the polyglutamine sequence in the transgenic animals of the invention is conferred by a constitutive, regulatable or tissue specific expression control element. In one aspect, a tissue specific expression control element confers neural, retinal, muscle or mesoderm cell expression. In another aspect, a tissue 5 specific expression control element comprises an *Appl* or *rhodopsin 1* promoter or GLASS transcription factor element.

Transgenic animals of the invention further include animals having a polyglutamine sequence of sufficient length to produce toxicity in one or more cells, tissue or organs of the animal. In one embodiment, toxicity is produced in a neuron cell or brain. In another 10 embodiment, toxicity is produced in a retinal cell or eye. In additional embodiments, toxicity is produced in muscle and mesoderm. Such animals can further include a gene that increases or decreases polyglutamine toxicity produced in the cell, tissue or organ. In one embodiment, such an animal includes a marker sequence inserted into its genomic DNA, wherein the marker is located adjacent to a gene or inserted into a gene whose expression or 15 activity increases or decreases polyglutamine toxicity in the animal. In one aspect, the marker sequence is near or inserted into a gene containing a J domain. In another aspect, the marker sequence is near or inserted into HDJ1. In yet another aspect, the marker sequence is near or inserted into TPR2. In still another aspect, the marker sequence is near or inserted into MLF gene.

20 Thus, methods for identifying a compound or transactivating factor that modulates polyglutamine toxicity in an animal also are provided. In one embodiment, a method includes contacting an animal that exhibits polyglutamine toxicity with a test compound; and determining whether the test compound increases or decreases polyglutamine toxicity in the animal. Increased or decreased polyglutamine toxicity identifies the test compound as a 25 compound that modulates polyglutamine toxicity. The compound may be present in the animal's food or drink or administered to a tissue or organ of the animal (directly or indirectly).

In addition, methods of producing a transgenic animal characterized by 30 polyglutamine toxicity are provided. In one embodiment, a method includes transforming an animal embryo or egg with a transgene comprising a plurality of CAA and CAG sequences encoding a polyglutamine sequence having sufficient length to produce polyglutamine

toxicity in the animal produced from the embryo or egg; and selecting an animal that exhibits polyglutamine toxicity in one or more cells or tissues. Polyglutamine sequences need only be of a length (or sequence where other non-glutamine residues are present) to produce toxicity in one or more cells, tissue or organs of the animal. Animal produced by these 5 methods include transgenic animals of the invention.

Compositions including isolated polynucleotides and polypeptides are also provided. In one embodiment, a polypeptide or a polynucleotide encodes a polypeptide that decreases polyglutamine toxicity. In one embodiment, a polynucleotide sequence has about 65% or more identity to a *Drosophila* TPR2 (dTPR2) sequence set forth as SEQ. ID NO:2, with the 10 proviso that the sequence is distinct from the EST sequences set forth in Figure 11. In another embodiment, a polynucleotide sequence has about 65% or more identity to a *Drosophila* MLF (dMLF) sequence set forth as SEQ. ID NO:4, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 12. Functional subsequences of TPR2 and MLF that decrease polyglutamine toxicity also are provided.

15 Invention polynucleotides can be operatively linked to an expression control element. In one embodiment, an expression control element confers expression in a cell, organ or tissue that has or is at risk of having polyglutamine toxicity. In one aspect, an expression control element confers expression in neuron, eye, muscle or mesoderm. In additional aspects, an expression control element is an *Appl* or *rhodopsin 1* promoter or GLASS 20 transcription factor element.

Further provided are isolated polynucleotide sequences that to invention *Drosophila* TPR2 (dTPR2) set forth as SEQ. ID NO:2, and dMLF set forth as SEQ. ID NO:4, sequences. In one embodiment, a sequence hybridizes to a *Drosophila* TPR2 (dTPR2) sequence set forth as SEQ. ID NO:2 under moderately stringent or highly stringent conditions, with the proviso 25 that the sequence is distinct from the EST sequences set forth in Figure 11. In another embodiment, a sequence hybridizes to a *Drosophila* MLF (dMLF) set forth as SEQ. ID NO:4 under moderately stringent or highly stringent conditions, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 12.

Such polynucleotide sequences can be of any length, and include, *inter alia*, 30 polynucleotide having 20 or more contiguous nucleotides, polynucleotide having 30 or more

contiguous nucleotides, polynucleotide having 40 or more contiguous nucleotides, polynucleotide having 50 or more contiguous nucleotides, etc.

Such sequences further include sequences that encode polypeptides, including functional polypeptides as described herein. In one embodiment, a sequence encodes a 5 subsequence of TPR2 that decreases polyglutamine toxicity. In another embodiment, a sequence encodes a subsequence of MLF that decreases polyglutamine toxicity. Expression of such sequences can be conferred by an expression control element, for tissue specific expression, for example. Polypeptides encoded by such sequences also are provided.

Compositions of the invention further include mammalian (e.g., human) homologues 10 of the genes that modulate polyglutamine toxicity in an animal as described herein operatively linked to an expression control element in a pharmaceutically acceptable carrier. In one embodiment, a composition includes a polynucleotide sequence encoding a human MLF polypeptide operatively linked to an expression control element in a pharmaceutically acceptable carrier. In another embodiment, a composition includes a polynucleotide 15 sequence encoding a human TPR2 polypeptide operatively linked to an expression control element in a pharmaceutically acceptable carrier. In additional embodiments, expression control elements confer expression of the mammalian (e.g., human) homologue in a cell, tissue or organ of a subject, having or at risk of having polyglutamine toxicity or a polyglutamine related disorder, as described herein.

20 Methods of identifying compounds or trans-activating protein factors that modulate expression or activity of a target dHDJ1, dTPR2 and dMLF also are provided. In one embodiment, a target gene is screened by transforming host cells with a promoter or regulatory region of the target gene operatively linked to a reporter construct. In various aspects, a promoter or regulatory region of the target gene includes a sequence set forth in 25 any of SEQ ID NO:s:9, 10 or 11. Candidate target gene promoters and regulatory regions also include promoter or regulatory regions of mammalian (e.g., human) homologues of dHDJ1, dTPR2 and dMLF.

In another embodiment, a method includes incubating components containing HDJ1, 30 TPR2 and MLF polypeptide or subsequence thereof, or a cell or animal expressing HDJ1, TPR2 and MLF polypeptide or subsequence thereof, and a test compound, under conditions sufficient to allow the components to interact. The effect of the test compound on HDJ1,

TPR2 and MLF polypeptide activity (e.g., polyglutamine toxicity) or expression is then determined.

In yet another embodiment, transactivating factors are identified using the polynucleotides of the invention *in vitro* or in a cell-based assay. A method includes 5 contacting a promoter or regulatory region of a target gene of HDJ1, TPR2 or MLF (e.g., a sequence set forth in any of SEQ ID NO:s:9, 10 or 11) with a candidate factor and determining whether the factor binds to the promoter or regulatory region. The invention methods therefore include *in vitro*, cell-based and *in vivo* methods to screen for effector compounds, transacting factors or binding proteins. Such methods are useful for identifying 10 transactivating factors or other compounds that modulate HDJ1, TPR2 or MLF expression and are therefore applicable in methods of identifying treatments as well as the treatment methods described herein.

Methods of increasing survival of a cell having or at risk of having polyglutamine toxicity are also provided. In one embodiment, a method includes contacting the cell with an 15 amount of TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding TPR2 or MLF polypeptide, to increase survival of the cell. Such methods include *in vitro*, *ex vivo* and *in vivo*, and where the cell is a neural, retinal, muscle or mesoderm cell.

Methods of decreasing apoptosis of a cell also are provided. In one embodiment, a method includes contacting the cell with an amount of TPR2 or MLF polypeptide sequence 20 or a polynucleotide sequence encoding TPR2 or MLF polypeptide to decrease apoptosis of the cell. Such methods include *in vitro*, *ex vivo* and *in vivo*, and where the cell is a neural, retinal, muscle or mesoderm cell.

Methods of decreasing polyglutamine toxicity in a cell having or at risk of having 25 also are provided. In one embodiment, a method includes contacting the cell with an amount of J domain containing polypeptide, TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide sequence to decrease polyglutamine toxicity in the cell. The toxicity may be decreased by decreasing cell death or apoptosis. The toxicity may be decreased by decreasing protein aggregation, increasing transport or folding, etc.

Such *in vitro*, *ex vivo* and *in vivo* methods include where the cell is a neural, retinal, 30 muscle or mesoderm cell. Thus, methods of decreasing polyglutamine toxicity in a tissue or

organ of a subject having or at risk polyglutamine toxicity also are provided. In one embodiment, a method includes contacting the cell, tissue or organ with an amount of a J domain containing polypeptide, a TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, to decrease polyglutamine toxicity in the cell, tissue or organ of the subject. In various aspects, the tissue is brain, eye, muscle or mesoderm.

Methods of decreasing the severity of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in a subject having or at risk of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder are provided. In one embodiment, a method includes administering to the subject an amount of J domain containing polypeptide, a TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, to decease the severity of the frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in the subject.

Methods of treatment include prophylactic administration. Disorders treatable include neurological and muscle disorders and disorders that impair long term or short term memory or coordination of the subject. Disorders treatable also include disorders characterized by the presence of protein aggregates, amyloid plaques, degeneration or atrophy in an affected tissue or organ.

Particular disorders treatable by the methods of the invention include Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD), Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma. The severity is decreased by decreasing cell death or apoptosis, increasing cell survival, decreasing protein aggregation, increasing protein folding, transport, etc. Severity is also decreased by slowing the progression or reversing one or more symptoms of the disorder (e.g., decreasing memory loss, improving memory, decreasing loss of coordination, improving coordination).

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the (A) polynucleotide and (B) encoded polypeptide sequences containing polyglutamine tracts of 20 and 127 amino acids and a hemagglutinin tag with the amino acid residues flanking the polyglutamine repeats. Underlining indicates the coding region of the polynucleotide sequence and italics indicates the Kozak sequence.

Figure 2 is a diagram showing P-element expression constructs encoding variously sized hemagglutinin (HA)-tagged polyglutamine sequences. (A) contains the full length *prospero* gene linked to the indicated HA-tagged polyglutamine encoding sequences located towards the 3' end; (B) contains a partial cDNA sequence encoding 422 amino acids of the C-terminus of *prospero* linked to variously sized HA-tagged polyglutamine encoding sequences; (C) contains variously sized HA-tagged polyglutamine encoding sequences; (D) contains variously sized HA-tagged polyglutamine encoding sequences driven by one, two or five eye-specific GLASS response elements (1GR, 2GR and 5GR). Polyglutamine tract sizes are denoted as 20, 41, 63, 127, 190 and 223 CAGs. UAS indicates the position of the upstream activating sequence that is responsive to the yeast GAL4 transcription factor. Miniwhite gene produces red pigmentation in the eye.

Figure 3 is a schematic diagram showing a genetic scheme for generating P-element mutants, screening for modulators of polyglutamine toxicity by crossing a fly that exhibits polyglutamine toxicity with the P-element mutants and isolating a modulatory P-element insertion on chromosome 3. EP55 (virgin females): source of transposable P-element; P[Δ 2-3]: source of transposase; F: female; M: male; CyO: balancer chromosome 2; TM3: balancer chromosome 3. Xa: translocation (2;3) Xa. (Chromosome 4 is omitted).

Figure 4 shows structural and histological changes that occur after expressing 127Q in the eye and suppression of the toxic effect by EU3500 P-element, dHDJ1 cDNA, EU3220 P-element, and dTPR2 cDNA. (A) Control expressing GAL4 regulated by GMR in the absence of 127Q; (B) flies expressing 127Q peptide driven by GMR-GAL4; (C) suppressor P-element insertion EU3500 restores external eye structure and pigmentation despite presence of polyglutamine aggregates; (D) confirmation of suppression in flies carrying a transgenic insertion of dHDJ1 cDNA, corresponding to the gene downstream of the EU3500 P-element insertion; (E) suppressor P-element insertion EU3220 improves external eye

structure and pigmentation; **(F)** confirmation of suppression in flies carrying a transgenic insertion of dTPR2 cDNA, corresponding to the gene downstream of the EU3220 P-element insertion. SEM = Scanning electron microscopy. FITC = Frozen eye sections labeled with Ab to the HA tag on 127Q peptide (green). FITC+DAPI = Double exposure with DAPI to stain nuclei (blue).

Figure 5 shows structural and histological changes that occur after expressing 127Q in the eye and suppression of the toxic effect by dMLF. **(A)** Control in the absence of 127Q, expressing GAL4 regulated by GMR, the eye-specific enhancer/promoter; **(B)** flies expressing 127Q peptide driven by GMR-GAL4; **(C)** suppressor P-element insertion EU2490 partially restores external eye structure and pigmentation; **(D and E)** flies carrying a transgenic insertion of dMLF cDNA, corresponding to the gene downstream of the EU2490 P-element insertion, either on chromosome 2 or on chromosome 3, as indicated, confirm the identity of the suppressor gene; **(F)** double dosage of dMLF expression, achieved by combining both the chromosome 2 and chromosome 3 transgenes. Abbreviations are as above.

Figure 6 shows a sequence alignment between *Drosophila* HDJ1 (dHDJ1) and human HDJ1 (hHsp40/HDJ1). Overall amino acid sequence homology is 54% identical (dark gray) and 72% similar (light gray). J region homology (bold underlining) is 74% identical (dark gray) and 88% similar (light gray).

Figure 7 shows a sequence alignment between *Drosophila* dTPR2 and the human tetratricopeptide repeat protein 2 (hTPR2). Overall amino acid sequence homology is 46% identical and 67% similar, denoted as above. J region homology (bold underlining, from about amino acid 401 to 469) is 74% identical and 93% similar, denoted as above. Arrows indicate the seven tetratricopeptide repeats (TPR₁ approximately amino acids 45-82; TPR₂ approximately amino acids 83-116; TPR₃ approximately amino acids 117-150; TPR₄ approximately amino acids 231-264; TPR₅ approximately amino acids 277-310; TPR₆ approximately amino acids 315-348; and TPR₇ approximately amino acids 349-382).

Figure 8 shows a sequence alignment between *Drosophila* myeloid leukemia factor 1 (dMLF) and its human homologue (dMLF). Overall amino acid sequence homology is 32% identical and 49% similar, denoted as above. The region absent from the full dMLF protein in the EU2490 P-element flies (MSLF....GLMN) which exhibit suppression of

polyglutamine toxicity is indicated by an arrow pointing to the left. The portion of hMLF included in the chimeric NPM-MLF created by the (3:5)(q25.1,q34) chromosomal translocation (Yoneda-Kato *et al.*, *Oncogene*, **12**:265-275 (1996)) is indicated by an arrow pointing to the right. The segment of hMLF in NPM-MLF required for its proapoptotic activity (Yoneda-Kato *et al.*, *Oncogene*, **18**:3716-3724 (1999)) is indicated by a gray bar.

5 **Figure 9** shows an **(A)** amino acid and **(B)** nucleic acid sequence encoding *Drosophila* TPR2 (dTPR2), set forth as SEQ ID NO:1 and SEQ ID NO:2, respectively.

10 **Figure 10** shows an **(A)** amino acid and **(B)** nucleic acid sequence encoding *Drosophila* MLF (dMLF), set forth as SEQ ID NO:3 and SEQ ID NO:4, respectively.

15 **Figure 11** shows a nucleic acid sequence alignment between *Drosophila* dTPR2 and several ESTs.

20 **Figure 12** shows a nucleic acid sequence alignment between *Drosophila* dMLF and several ESTs.

25 **Figure 13** shows an **(A)** amino acid and **(B)** nucleic acid sequence encoding human TPR2, set forth as SEQ ID NO:5 and SEQ ID NO:6, respectively.

30 **Figure 14** shows an **(A)** amino acid and **(B)** nucleic acid sequence encoding human MLF, set forth as SEQ ID NO:7 and SEQ ID NO:8, respectively.

35 **Figure 15** is a drawing of a plasmid useful for drug screening.

40 **Figure 16** shows a polynucleotide sequence located 5' of a nucleic acid sequence encoding dHDJ1, set forth as SEQ ID NO:9.

45 **Figure 17** shows a polynucleotide sequence located 5' of a nucleic acid sequence encoding dTPR2, set forth as SEQ ID NO:10.

50 **Figure 18** shows a polynucleotide sequence located 5' of a nucleic acid sequence encoding dMLF, set forth as SEQ ID NO:11.

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DETAILED DESCRIPTION OF THE INVENTION

60 The present invention provides an *in vivo* animal model that mimics the cellular degeneration observed in human neurological disorders. A genetic screen in *Drosophila* that exhibits toxicity in response to expression of expanded polyglutamine sequences was used to identify genes that modulate polyglutamine toxicity. Using the model, lines that contained either suppressors or enhancers of toxicity were produced. Of the suppressors, three genes

were identified that decrease polyglutamine toxicity; a *Drosophila* homologue of human HDJ1 (dHDJ1), a *Drosophila* homologue of human TPR2 (dTPR2); and a *Drosophila* homologue of human myeloid leukemia factor 1 (dMLF). Expression of each of these cDNAs in the animal model ameliorates the toxicity conferred by expanded polyglutamine repeat sequences both in the eye and in neurological tissues. The *in vivo* animal model system is therefore useful in discovering genes and other compounds with therapeutic applications in polyglutamine disorders, frontotemporal dementia, prion diseases and protein aggregation disorders. Particular therapeutic applications include, for example, treating Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD), Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma.

Thus, in accordance with the present invention, there are provided methods for screening for genes and other compounds that modulate polyglutamine toxicity. In one embodiment, a method of the invention includes providing a first animal expressing a polyglutamine sequence that produces polyglutamine toxicity in the animal; breeding the first animal to a second animal, wherein the second animal has a marker sequence inserted into its germline, thereby producing progeny; screening the progeny for increased or decreased polyglutamine toxicity relative to the first animal thereby identifying a progeny having increased or decreased polyglutamine toxicity; and identifying one or more genes adjacent to the marker sequence or having an insertion of the marker sequence that confers increased or decreased polyglutamine toxicity in the progeny. In another embodiment, a method of the invention further includes identifying a mammalian homologue (e.g., human) of the gene that confers increased or decreased polyglutamine toxicity. Identification of such homologues can be performed by comparison to sequence databases (Genbank, Swiss-prot, EMBL, etc.), including the complete human sequence database (Celera Genomics, Inc., Rockville, MD). Alternatively, library screening of cDNA, genomic or expression libraries can be performed using libraries available in the art.

As used herein, the term "animal" refers to a multicellular organism that reproduces sexually and that exhibits one or more characteristics of polyglutamine toxicity when a polyglutamine repeat sequence of sufficient length is expressed in a cell or tissue of the

organism. As such sequences produce polyglutamine toxicity in a wide variety of animals, including human and non-human mammals (*e.g.*, bovine, murine, porcine, ungulates, etc.), many different types of non-human animals are applicable in the screening methods of the invention. In one embodiment, the animal comprises an invertebrate. Preferred invertebrate 5 animals are insects, such as flies, *e.g.*, of the genus *Drosophila*. In another embodiment, the animal comprises *Caenorhabditis elegans*. The exemplified animal is of the species *Drosophila melanogaster*.

As used herein, the term “modulate,” means an increase, decrease or alteration of the term modified. For example, the term modulate can be used in various contexts to refer to a 10 morphological or structural condition of a cell or tissue, a physiological condition of an organism, or an activity, a function, activity or expression of a polypeptide, gene or signaling pathway. Thus, where the term “modulate” is used to modify the term “polyglutamine toxicity,” this means that the toxicity is either increased (worsens) or decreases (improves). Detecting increased or decreased polyglutamine toxicity can be determined as set forth herein 15 using an *in vivo* animal model. For example, improvement in cell and tissue morphology or structure, eye pigmentation or aberrant behavior, animal survival or development, or decreased protein aggregates, of the *Drosophila* animal model, indicates decreased polyglutamine toxicity whereas a worsening of one or more of these parameters indicates increased polyglutamine toxicity.

20 The polyglutamine sequences will typically contain consecutive glutamine residues (Q_n). Polyglutamine sequences that produce toxicity in a cell or tissue will have a sufficient number of glutamine residues to produce toxicity. Such toxic sequences typically are at least about 30 glutamine residues or greater in length, although they may be less in particularly sensitive animals, cells or tissues, or where a non-polyglutamine sequence that enhances 25 toxicity of the polyglutamine sequence is also included. Toxic sequences, for example, can be between about 30 and 40, 40 and 50, 50 and 60, 60 and 70, 70 and 80, 80 and 90, 90 and 100, 100 and 110, 110 and 120, 120 and 130, 130 and 140, 140 and 150, etc. Such sequences will likely be between about 50 and 75, 75 and 100, 100 and 125, 100 and 150, or greater 30 (150 and 200, 200 and 250, 250 and 300, 300 and 500, etc.). Non-toxic sequences, which are useful as a control or to detect increased sensitivity to polyglutamine toxicity, will typically be shorter, for example, between about 5 and 10, 10 and 20, 20 and 30, 5 and 20 amino acids

in length, or greater, where such sequences may not be toxic in certain tissues, even though they may be toxic in others. The glutamine residues in the repeat sequences need not be consecutive. For example, the glutamines can have one or more non-glutamine residues interspersed within the glutamine repeat (*e.g.*, Q_nX_nQ_n, where X is a non-glutamine residue, and n is any integer between 1 and 150). For toxic sequences, such interspersing non-glutamine residues may or may not have an affect on toxicity. Accordingly, toxic polyglutamine sequences that have non-glutamine residues are also included in the polyglutamine repeat sequences described herein. The effect of non-glutamine residues on toxicity can be determined using *in vitro*, cell based assays or *in vivo* toxicity assays described herein or known in the art (*e.g.*, *in vivo* animal assays that detect cell/tissue degeneration, death or apoptosis, behavioral abnormalities, altered development or viability, or protein aggregate formation; *in vitro* assays that detect protein aggregation or misfolding; and cell based assays that detect aggregates in nucleus or in the cytoplasm, or extracellular aggregates such as plaques, etc.).

Polyglutamine repeat sequences expressed in the animal will be encoded by either a plurality of CAG or CAA codons or a combination of CAGs and CAAs. Where the sequence is encoded by a combination of CAGs and CAAs, the ratio of the number of CAGs to CAAs can be from about 240:1, 210:1, 180:1, 150:1, 120:1, 90:1, 75:1, 60:1, 45:1, 30:1, 15:1, 9:1, 3:1, 1:1, or less, for example, 1:3, 1:9, 1:15, 1:30, 1:45, 1:60, 1:75, 1:90, 1:120, 1:150, 1:180, 1:210, 1:240, or even less. The presence of one or more CAAs in a plurality of CAGs encoding a polyglutamine repeat sequence decreases the likelihood that sequence truncations will occur. For longer polyglutamine repeat sequences, for example, those greater than about 40, 50, 60, 70, 80, 90, 100, 110, 120 or more glutamine residues, which typically produce polyglutamine toxicity, the effect increases as the length of the sequence increases. Thus, including one or more CAAs within a sequence of CAGs can lead to expression of an encoded polyglutamine sequence that does not become truncated. Accordingly, in the transgenic animals of the invention that include a polyglutamine sequence of sufficient length to produce toxicity, it is likely that at least one CAA will be included with a plurality of CAGs encoding the sequence. The CAAs in the polynucleotide encoding the polyglutamine repeat sequence can be interspersed at regular or irregular intervals within the polynucleotide, for example, a single CAA within a CAG repeat encoding 40-50 amino

acids, 30-40 amino acids, 20-30 amino acids, 10-20 amino acids or 5-10 or fewer amino acids. Of course, the sequence can have greater numbers of CAAs than CAGs, if desired.

As used herein, the term "marker" or "marker sequence" means a sequence that is "marked" so as to be identifiable. The presence of the marker in the genome of the organism allows identification of gene(s) that modulate toxicity. Detecting the presence of a polynucleotide marker sequence in the genome of the organism, and genes that modulate toxicity, can be performed by sequence analysis using marker specific primers, for example. Thus, when using a polynucleotide sequence marker, it will typically be distinguishable from endogenous gene sequences so that the marker may be sequenced without interference from endogenous gene sequences.

Where a marker sequence comprises a polynucleotide sequence inserted into the genome of the animal, the inserted sequence may alter expression or activity of one or more genes near the sequence. Where the animal having the marker exhibits a modulation of polyglutamine toxicity, the effect will therefore be due to changes in expression or activity of the gene(s) near or adjacent to the marker sequence, or a gene into which the marker has been inserted. The latter will typically result in decreased expression of the gene, or an altered or aberrant activity, due to the marker disrupting the sequence of the endogenous gene, such as by insertion into the coding sequence (producing a deleted or "Knocked-out" gene, or a truncated gene product, etc.) or insertion into a 5', 3' or intron regulatory sequence that confers expression of the endogenous gene. An insertion of a marker can also produce a gene product that lacks a portion of the sequence, or contains a foreign sequence encoded by the marker. A marker that is positioned near a gene, but not inserted, likely alters expression levels of the endogenous gene (increasing or decreasing).

Thus, in one embodiment, a marker sequence decreases expression of an endogenous gene. In another embodiment, a marker sequence increases expression of an endogenous gene. In yet another embodiment, a marker sequence alters an activity of an endogenous gene (increases or decreases).

Decreased polyglutamine toxicity will occur when genes that increase polyglutamine toxicity are disrupted or their expression is decreased, or when expression or activity of a suppressor of toxicity is increased. Decreased polyglutamine toxicity will result in improvements in the phenotype associated with toxicity (*e.g.*, a return to a more normal cell

morphology or tissue structure, increased eye pigmentation, decreased animal lethality or behavioral abnormalities, normal development, decreased protein aggregation, increased cell survival, decreased apoptosis, increased cell proliferation/differentiation etc.), or a decreased sensitivity to expansion of polyglutamine repeat sequences.

5 Increased polyglutamine toxicity will occur when genes that decrease polyglutamine toxicity are disrupted or their expression is decreased, or when expression or activity of an enhancer of toxicity is increased. Increased toxicity will result in more pronounced toxicity or a worsening of the phenotype associated with toxicity (*e.g.*, a more pronounced degeneration of cell morphology or loss of characteristic tissue structure, loss of eye
10 pigmentation, increased animal lethality or behavioral abnormalities, increased protein aggregation, decreased cell survival, increased apoptosis, decreased cell proliferation/differentiation, etc.), or an increased sensitivity to shorter polyglutamine sequences. For example, a 20 residue glutamine repeat sequence that is normally non-toxic in the animal may be toxic when the marker sequence decreases or disrupts expression or
15 alters activity of a toxicity suppressor, or increases expression or alters activity of a toxicity enhancer.

As discussed, marker sequences need only be distinguishable from endogenous genes in order to identify one or more nearby genes that modulate polyglutamine toxicity. In one embodiment, a marker comprises a P-element. In another embodiment, the marker further includes an expression control element regulating expression of one or more genes nearby the marker. In one aspect, the expression control element increases expression of one or more of the nearby genes. In another aspect, the expression control element decreases expression of one or more of the nearby genes. In additional aspects, the expression control element is regulatable (inducible or repressible) or tissue specific.
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As used herein, the term “expression control element” means an element that influences expression of a nearby or adjacent gene(s) sequence to which it is operatively linked. An expression control element operatively linked to a nucleic acid sequence controls transcription and, as appropriate, translation of the nucleic acid sequence. Thus an expression control element can include one or more promoters, enhancers, transcription terminators, a start codon (*e.g.*, ATG) in front of a protein-encoding gene. “Operatively linked” refers to a juxtaposition wherein the components so described are in a relationship
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permitting them to function in their intended manner. Expression control elements either increase, decrease or confer regulatable (inducible expression or repression) expression of a nearby or adjacent gene(s). For example, where the animal expresses or is made to express a transcriptional activator that is present in a wide variety of cell types, *i.e.*, it is constitutively expressed, an expression control element that responds to the transcriptional activator can be used to increase expression of the nearby or adjacent gene in the cells in which the activator is present. Where the animal expresses a transcriptional repressor, an expression control element that responds to the transcriptional repressor can be used to decrease expression of the nearby or adjacent gene in the cells in which the activator is present.

10 Expression control elements also include elements that confer tissue or cell specific expression, such as in eye, neural, muscle or mesoderm. For example, the GLASS sequence, a segment of the *rhodopsin 1* regulatory region, confers expression in *Drosophila* retinal cells. The *Appl* control element confers expression in neural cells. Other elements that confer tissue or cell specific, including muscle and mesoderm elements, are known or can 15 otherwise be identified using methods known in the art.

Expression control elements that may also be used include those that are normally not present in the organism. For example, the yeast GAL4 responsive expression control element, UAS, is normally not present in animals yet is activated when driven by the yeast 20 GAL4 protein. A GAL4 driven UAS element can be used to express a polyglutamine sequence transgene in response to GAL4 in a transgenic animal or to express a nearby or adjacent gene when included with a marker sequence. A tetracycline response element can be used to confer conditional expression in various tissues. Accordingly, a variety of expression control elements, as well as combinations and/or multiples of such elements, (*e.g.*, UAS and GR, see Figure 2) can be used for expression of the polyglutamine sequences or to 25 alter expression of a nearby or adjacent gene(s) in the animals that include a marker sequence.

As used herein, the terms “near” “nearby” or “adjacent,” when used to describe the position of a marker sequence inserted into the animal’s genome in relationship to a gene, means that the marker is close enough to the gene(s) to either affect activity or expression. 30 Typically, a marker that does not include an expression control element, to effect expression or activity, will either be inserted into the coding sequence of the gene or an intron, or 5' or

3' sequence thereby controlling expression of the gene, transcript stability, splicing of the transcript, etc. Such markers will generally be within about 5 Kb or less of the gene, depending on the nature of the genes' regulatory region. Markers that further include expression control elements, such as an enhancer that can act at a distance, up to 50 Kb, can 5 be much farther away from the gene and still affect activity or expression of the gene. More typically, a marker will be within 5 Kb or less of the gene coding sequence (*e.g.*, less than 4 Kb, 3 Kb, 2Kb, 1 Kb, 0.5Kb, 250bp, 100 bp, 50 bp, etc.). The type and number of expression control elements included with the marker will determine the amount of expression control exerted over the gene(s), and the distance from the gene(s) with which it will exert control.

10 In order to produce progeny having increased or decreased polyglutamine toxicity relative to the first animal that exhibits polyglutamine toxicity, at least one marker sequence will be present in the germline of the second animal. Typically, second animals will each have one or a few marker sequences inserted into the germline so that the gene(s) that confers altered polyglutamine toxicity in progeny will be easier to identify. Nevertheless, multiple 15 marker sequences can be present in a given second animal without departing from the invention. In the case of multiple markers located at different positions within the genome of the second animal, genes near or adjacent to each of the marker (or having insertions of the marker) can be individually tested for activity by individually expressing each of the genes, for example, in a transgenic animal that exhibits polyglutamine toxicity or a cell-based or 20 *in vitro* assays that reflects one or more aspects of toxicity (*e.g.*, protein aggregation, misfolding aberrant transport, etc.).

The greater the number of second animals that can be screened, each of which differs as to the location of the marker inserted into their genome, the greater the number of candidate modulatory genes that can be screened. Thus, by screening a sufficient number of 25 animals having marker sequences inserted randomly throughout their genome, for example, every gene in the animal can be tested for its modulation of polyglutamine toxicity. Accordingly, a population of second animals, for example, 10 to 100, 100 to 500, 500 or more, *e.g.*, 1000 or more, 5000 or more, or enough animals to encompass the entire number of genes of the animal can be screened. In the present case, 7000 *Drosophila* having 30 randomly generated P-element insertions were screened for modulators of polyglutamine toxicity identified 30 enhancers and 29 suppressors of polyglutamine toxicity. It is

anticipated that approximately 50,000 *Drosophila* each having a randomly generated P-element insertion would be sufficient to screen the entire *Drosophila* genome for modulators of polyglutamine toxicity.

Non-*Drosophila* genes can also be assayed for the ability to modulate polyglutamine toxicity. *Drosophila* exhibiting polyglutamine toxicity engineered to contain non-*Drosophila* gene sequences can be used to screen for gene sequences from other organisms that modulate toxicity. For example, a P-element containing a mammalian (e.g., human) gene can be introduced into *Drosophila* exhibiting polyglutamine toxicity in order to screen the mammalian (e.g., human) gene for modulatory activity. Conceivably, a library of P-elements containing a library of any non-*Drosophila* organism genetic elements could be tested in order to directly identify genes of the non-*Drosophila* organism that modulate polyglutamine toxicity. Thus, a library of P-elements each containing a human gene, individually or as collections, can be introduced into *Drosophila* exhibiting polyglutamine toxicity in order to directly identify human genes that modulate polyglutamine toxicity. Accordingly, it is specifically intended that the methods of the invention include screening of non-*Drosophila* genes for their ability to modulate polyglutamine toxicity.

In the screening methods of the invention for identifying genetic elements that modulate polyglutamine toxicity, or polyglutamine related or like disorders, genetically manipulatable animals are preferred. Such animals are useful for introducing marker sequences at different locations within the animals' genome in order to test a variety of genes. An exemplary animal is of the genus *Drosophila*, in particular, *Drosophila melanogaster*. Marker sequences, in particular, random P-element insertions in the genome were generated in *Drosophila* as outlined in Figure 3. The F2 males having colored eyes (indicating the presence of the P-element miniwhite gene as shown in Figure 2) were selected as they have a stable P-element insertion in their genome. Subsequent crosses between the F2 males and the *Drosophila* lines exhibiting polyglutamine toxicity produced progeny that exhibited altered polyglutamine toxicity.

As the methods of the invention for screening for genes and other compounds that modulate polyglutamine toxicity produce progeny in which toxicity is modulated in comparison to a parent, the invention further provides progeny animals produced by the methods of the invention. In one embodiment, a progeny exhibits increased polyglutamine

toxicity in comparison to a parent. In another embodiment, a progeny exhibits decreased polyglutamine toxicity in comparison to a parent. In still another embodiment, a progeny exhibits altered cell death or survival, apoptosis, proliferation, differentiation, behavior, development or viability, neuron excitability, protein aggregation (intracellular, in nucleus or 5 in cytoplasm, or extracellular), folding, transport or degradation, relative to a parent animal. The progeny that exhibit increased or decreased toxicity, cell death or survival, apoptosis, proliferation, differentiation, altered behavior, neuron excitability, development or viability, protein aggregation (intracellular, in nucleus or in cytoplasm, or extracellular), protein folding, transport or degradation, etc., relative to parent, are useful in further characterizing 10 the molecular aspects of the pathways of polyglutamine toxicity and disorders associated with cell death or survival, apoptosis, proliferation, differentiation, behavior, development or viability abnormality, protein aggregation (intracellular, in nucleus or in cytoplasm, or extracellular), folding, transport or degradation, in general, and the role of particular enhancers and suppressors in disease pathways associated with these characteristics.

15 In accordance with the present invention, there are also provided transgenic animals comprising one or more transgenes. In one embodiment, a transgenic animal of the invention includes a transgene containing a plurality of CAGs and at least one CAA encoding a polyglutamine repeat sequence. In one aspect, the polyglutamine repeat sequence is of a sufficient length or sequence to produce polyglutamine toxicity in one or more tissue or 20 organs of the transgenic animal. In another embodiment, a transgenic animal includes a marker sequence inserted into its genome, wherein the marker is located adjacent to a gene or inserted into a gene whose expression or activity increases or decreases polyglutamine toxicity in the animal. In one aspect, the marker sequence is near or inserted into a gene containing a J domain. In another aspect, the marker sequence is near or inserted into 25 HDJ1 gene. In yet another aspect, the marker sequence is near or inserted into a TPR2 gene. In still another aspect, the marker sequence is near or inserted into a MLF gene.

In yet another embodiment, a transgenic animal of the invention includes a transgene identified by a method of the invention. In one aspect, the transgene comprises HDJ1, TPR2 or MLF, mammalian, human or *Drosophila*. In another aspect, a transgenic animal of the 30 invention includes a transgene identified by a method of the invention and a transgene

encoding a polyglutamine repeat sequence. In various aspects, a transgenic animal is an invertebrate (e.g., *Drosophila melanogaster*).

As discussed, the number of CAGs to CAAs in a polynucleotide encoding a polyglutamine repeat sequence can vary. In one embodiment, the number of CAGs to CAAs is in ratio of between about 1:1 and 2:1. In another embodiment, the number of CAG's to CAA's is in ratio of between about 2:1 and 5:1. In additional embodiments, the number of CAG's to CAA's is in ratio of between about 5:1 and 10:1, between about 10:1 and 30:1 between about 30:1 and 50:1 and between about 50:1 and 90:1.

The transgenic animals of the invention that include a polyglutamine repeat sequence or a transgene can include any of a variety of expression control elements. In one embodiment, polyglutamine sequence expression is conferred by a constitutive, regulatable or tissue specific expression control element. In another embodiment, transgene expression is conferred by a constitutive, regulatable or tissue specific expression control element.

To target polyglutamine toxicity to particular cells or tissue of the animal, tissue specific expression control elements that confer expression of polyglutamine repeat sequences can be used. In addition to modulating polyglutamine toxicity in the tissues that express the polyglutamine repeat sequences, expression control elements that confer tissue specific expression can be included in a marker sequence to target that particular tissue or to confer expression of a transgene that modulates toxicity or any of the other phenotypes described herein in a target tissue. In one embodiment, the tissue specific expression control element confers expression in a neural, retinal, muscle or mesoderm cell. In one aspect, the tissue specific expression control element comprises an *Appl* or *rhodopsin 1* promoter or GLASS transcription factor element.

Other animals may be used in the invention so long as polyglutamine toxicity can be produced in a cell, tissue or organ of the animal. Such animals may be less genetically manipulatable than *Drosophila*, but, nevertheless, owing to artificial or natural (e.g., polymorphic) identifiable sequences present in the animal they may be used to identify genetic modulators of polyglutamine toxicity because breeding the animal may produce a progeny having altered polyglutamine toxicity. For identifying non-genetic modulators of toxicity and polyglutamine related disorders, such as drugs or compounds (e.g., small organic molecules that are generally membrane permeable or can be modified or included in a

membrane permeable material), the organisms need not be genetically manipulatable as the animal is merely contacted with the drug or compound. Thus, it is contemplated that any non-human animal that exhibits polyglutamine toxicity is applicable for identifying modulators of polyglutamine toxicity.

5 Thus, in accordance with the present invention, there are also provided methods for identifying a compound that modulates polyglutamine toxicity in an animal. A method of the invention includes contacting an animal that exhibits polyglutamine toxicity with a test compound and determining whether the test compound increases or decreases polyglutamine toxicity in the animal. A test compound that increases or decreases polyglutamine toxicity is
10 identified as a compound that modulates polyglutamine toxicity. In one embodiment, the test compound is present in the animal's food or drink. In another embodiment, the test compound is administered to a tissue or organ of the animal. Compounds which decrease polyglutamine toxicity can be a broad spectrum inhibitor of cell or tissue degeneration, death or apoptosis, for example, and can be useful in various therapies including the therapeutic
15 methods of the invention.

As with the screening methods and genetic elements that modulate polyglutamine toxicity described herein, such screening methods and the compounds identified are useful in identifying therapeutics and for treating polyglutamine toxicity and polyglutamine related disorders. In addition, such compounds are also useful as therapeutics that modulate cell
20 death or survival, apoptosis, proliferation, differentiation, development or viability, behavior, neuron excitability, protein aggregation (intracellular, in nucleus or in cytoplasm, or extracellular), folding, transport or degradation, and diseases associated with these processes.

As used herein, the term "transgenic animal" refers to a non-human animal whose somatic or germ line cells bear genetic information received, directly or indirectly, by genetic
25 manipulation at the subcellular level, such as by nucleic acid microinjection or infection of an egg or embryo with recombinant virus. In the present context, a "transgenic animal" also includes progeny animals produced by mating of such genetically manipulated transgenic animals. Invention transgenic animals can be either heterozygous or homozygous with respect to the transgene, although it is likely that for identifying genetic modulators of
30 polyglutamine toxicity that germline transgenics will be used.

The term "transgenic" also includes any animal whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by transgenic technology to induce a gene knockout. The term "gene knockout" as used herein, refers to the disruption of a targeted gene *in vivo* with a loss of function achieved by any transgenic technology which 5 can produce an animal in which an endogenous gene has been rendered non-functional or "knocked out." The term "transgenic" further includes cells or tissues (*i.e.*, "transgenic cell," "transgenic tissue") obtained from a transgenic animal genetically manipulated as described herein.

As discussed, transgenic animals that contain the marker sequences will generally 10 have the markers integrated into the germline. Such animals having a marker integrated into germ cells have the ability to transfer the marker to progeny offspring. Although it is preferred that the transgene be integrated into the animal's chromosome, the present invention also contemplates the use of extrachromosomally replicating sequences, such as those similar to yeast artificial chromosomes, so long as they can be passed onto progeny.

15 The transgenic animals as set forth herein include insects. The term "insect" as used herein includes all insect species. The term "insect" further includes an individual insect in any stage of development.

Transgenic animals can be produced by methods known in the art. For transgenic insects, generally the transgene is introduced at an embryonic stage. For example, transgenic 20 insects can be produced by introducing into single cell embryos invention polynucleotides, either naked or contained in an appropriate vector, by microinjection, for example, which can produce insects by P-element mediated germ line transformation (see *e.g.*, Rubin *et al.*, *Science* **218**:348-353 (1982)). Totipotent or pluripotent stem cells transformed by microinjection, calcium phosphate mediated precipitation, liposome fusion, retroviral 25 infection or other means are then introduced into the embryo, and the polynucleotides are stably integrated into the genome. A transgenic embryo so transformed then develops into a mature transgenic insect in which the transgene is inherited in normal Mendelian fashion. Additional methods for producing transgenic insects can be found, for example, in O'Brockta *et al.*, *Insect Biochem. Mol. Biol.* **26**:739-753 (1996) and in Louleris *et al.*, *Science* **270**:2002-30 2005 (1995).

In a particular embodiment, developing insect embryos are infected with a virus, such as a baculovirus (*e.g.*, *Autographa californica* AcNPV), containing the desired polynucleotide, and transgenic insects produced from the infected embryo. The virus can be an occluded virus or a nonoccluded virus. A virus can be occluded by coinfection of cells 5 with a helper virus that provides polyhedrin gene function. The skilled artisan will understand how to construct recombinant viruses in which the polynucleotide is inserted into a nonessential region of the baculovirus genome. For example, in the AcNPV genome, nonessential regions include the p10 region (Adan *et al.*, *Virology* **444**:782-793 (1982)), the DA26 region (O'Reily *et al.*, *J. Gen. Virol.* **71**:1029-1037 (1990)), the ETL region (Crawford 10 *et al.*, *Virology* **62**:2773-2781 (1988)), the egt region (O'Reily *et al.*, *J. Gen. Virol.* **64**:1321-1328), among others.

Significant homology exists among particular genes of different baculoviruses and therefore, one of skill in the art will understand how to insert an invention polynucleotide into similar nonessential regions of other baculoviruses. Thus, for example, a polynucleotide 15 encoding a polyglutamine repeat sequence, or a genetic modulator of polyglutamine toxicity (*e.g.*, J domain protein or HDJ1, TPR2 or MLF polypeptide) may be placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter). Depending on the vector utilized, any of a number of suitable transcription and translation elements, including constitutive, inducible and conditional promoters, enhancers, transcription terminators, etc. may be used 20 in order to transcribe polynucleotides (sense or antisense) or express polypeptides.

Alternatively, a transgene containing a nucleic acid sequence disrupting expression of a J domain protein, HDJ1, TPR2 or MLF may not contain a promoter as the nucleic acid sequence need not be transcribed or translated to obtain a transgenic insect having a disrupted gene.

25 Thus, the invention provides methods for producing transgenic animals characterized by polyglutamine toxicity. A method of the invention includes transforming an animal embryo or egg with a transgene comprising a plurality of CAA and CAG sequences encoding a polyglutamine sequence having sufficient length to produce polyglutamine toxicity in the animal produced from the embryo or egg; and selecting an animal that exhibits polyglutamine 30 toxicity in one or more cells or tissues. Such methods can include introducing into the genome of the insect a nucleic acid construct including a disrupted gene, and obtaining a

transgenic insect having a disrupted nucleic acid sequence, such as a gene encoding a J domain protein, HDJ1, TPR2 or MLF.

The invention also provides methods for producing transgenic animals having transgenes that modulate polyglutamine toxicity. In one embodiment, a method of the 5 invention includes transforming an animal embryo or egg from an animal that exhibits polyglutamine toxicity with a transgene comprising a polynucleotide encoding a polyglutamine toxicity modulating polypeptide; and selecting an animal produced from the embryo or egg that exhibits modulated polyglutamine toxicity in one or more cells or tissues.

As the transgenic insects described herein having invention polynucleotides or 10 invention polypeptides may exhibit an altered sensitivity to polyglutamine toxicity or polyglutamine related disorders, such transgenic insects can be useful, for example, as biological tools to elucidate the signaling pathways that these genes participate in. As discussed, animals having modulated polyglutamine toxicity can mate with other animals in order to determine the effect of various genetic combinations on polyglutamine toxicity.

15 Substantially pure, isolated and recombinant polypeptides that modulate polyglutamine toxicity are provided. In one embodiment, the polypeptide comprises a dTPR2 polypeptide characterized as having a predicted molecular weight of about 58,000 Da (58 kDa). dTPR2 polypeptide is exemplified by the 508 amino acid sequence set forth in SEQ ID NO:1 (Figure 9). In another embodiment, the polypeptide comprises a dMLF 20 polypeptide characterized as having a predicted molecular weight of about 30,000 Da (30 kDa). dMLF polypeptide is exemplified by the 273 amino acid sequence set forth in SEQ ID NO:3 (Figure 10).

Characteristic features of TPR2 include, for example, a J domain located at 25 approximately amino acids 401 to 469, which binds to other proteins having secondary and tertiary structure (Figure 7). J proteins are implicated in preventing protein aggregation. TPR2 also has multiple tpr domains which are found in proteins involved in protein import, neurogenesis, stress response, and chaperone action. Characteristic features of MLF are based on the role of its human counterpart in cell survival and proliferation. In this regard, human 30 MLF is associated with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (*Weiss et al., Amer. J. Med. Genet.*, **89**:14-22 (1999)). In stable transfections of NIH3T3 mouse fibroblast cells with MLF cDNA, MLF antibody stained the cytoplasm,

whereas the NPM-MLF chimeric product was exclusively nuclear and nucleolar (Bergmann *et al.*, *Cell*, **95**:331-341 (1998). Neither MLF nor NPM alone had any detectable effect, but NPM-MLF induced apoptosis. The region necessary for apoptotic activity was narrowed down to a 92-amino acid stretch in MLF (Figure. 8) (Bergmann *et al.*, 1998, *supra*).

- 5 Therefore, it is likely that the corresponding region of dMLF has a similar role in modulating apoptosis. For example, dMLF may protect against polyglutamine toxicity through its function as a component of cell survival signaling pathway.

As used herein, the terms “peptide,” “polypeptide” and “protein” are used interchangeably and refer to two or more amino acids covalently linked by an amide bond or equivalent. The polypeptides of the invention are of any length and include L- and D-isomers, and combinations of L- and D-isomers. The polypeptides can include modifications typically associated with post-translational processing of proteins, for example, cyclization (e.g., disulfide bond), phosphorylation, glycosylation, carboxylation, ubiquitination, myristylation, or lipidation. Polypeptides described herein further include compounds having 10 amino acid structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues, so long as the mimetic has one or more functions or activities of a native polypeptide set forth herein. Non-natural and non-amide chemical bonds, and other coupling means can also be included, for example, glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, or N, N'-dicyclohexylcarbodiimide (DCC). Non-amide bonds can include, for example, ketomethylene aminomethylene, olefin, ether, thioether and the like (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, “Peptide and Backbone Modifications,” Marcel Decker, NY).

As used herein, the term “isolated,” when used as a modifier of polypeptide, means 25 that they are produced by the hand of man and are therefore separated from their native *in vivo* cellular environment. An “isolated” polypeptide, antibody or polynucleotide can also be “substantially pure” when free of most or all of the materials with which they may normally be associated with in nature. Thus, an isolated compound that also is substantially pure does not include polypeptides or polynucleotides present among millions of other 30 sequences, such as nucleic acids in a genomic or cDNA library, for example. Typically, the purity can be at least about 60% or more by mass. The purity can also be about 70% or 80%

or more, and can be greater, for example, 90% or more. Purity can be determined by any appropriate method, including, for example, UV spectroscopy, chromatography (*e.g.*, HPLC, gas phase), gel electrophoresis and sequence analysis (nucleic acid and peptide).

As used herein, the term “recombinant,” when used as a modifier of polypeptides, 5 polynucleotides and antibodies, means that the compositions have been manipulated (*i.e.*, engineered) in a fashion that generally does not occur in nature (*e.g.*, *in vitro*). A particular example of a recombinant polypeptide would be where HDJ1, TPR2 or MLF polypeptide is expressed by a cell transfected with a polynucleotide encoding the polypeptide. A particular example of a recombinant polynucleotide would be where a nucleic acid (*e.g.*, genomic or 10 cDNA) encoding HDJ1, TPR2 or MLF is cloned into a plasmid, with or without 5', 3' or intron regions that the gene is normally contiguous with in the genome of the organism.

Another example of a recombinant polynucleotide or polypeptide is a hybrid or fusion sequence, such as a chimeric sequence comprising HDJ1, TPR2 or MLF and a second sequence, such as a heterologous functional domain.

15 The invention further includes polypeptides having minor modifications of and additions to the amino acid sequence of the HDJ1, TPR2 and MLF polypeptides set forth herein. Such polypeptides have one or more activities or biological functions substantially equivalent to unmodified HDJ1, TPR2 and MLF polypeptide. Such activities include, for example, decreasing polyglutamine toxicity, increasing cell survival, decreasing 20 degeneration, cell death or apoptosis, decreasing protein aggregation, misfolding, plaque formation, improving development, viability, or behavior, etc.

Thus, a “functional polypeptide” or “active polypeptide” refers to a modified 25 polypeptide that possesses a function or biological activity identified through an assay. As described herein, a particular example of a biological activity is the ability to modulate (increase or decrease) polyglutamine toxicity *in vivo*. Another example of a biological activity is the ability to modulate cell death, apoptosis, survival, degeneration, protein aggregation, transport, folding, degradation, etc. Other examples include the ability to directly or indirectly decrease cellular toxicity associated with protein aggregation, or aberrant or undesirable protein folding, transport or degradation. Thus, functional assays such 30 as cell survival and cell death assays (*e.g.*, apoptosis), development or viability assays, behavioral assays, neuron excitability assays and protein binding, folding, aggregation and

transport assays, as well as toxicity in cells or in other organisms can be used to identify polypeptides having one or more functions described herein.

Cell-based assays for assaying toxicity (cell death, apoptosis and protein aggregation) are described, for example, in Hackam *et al.*, *Human Molecular Genetics* **8**:25-33 (1999) and

5 Saudou *et al.*, *Cell* **95**:55-66 (1998). Other animal assays include mouse behavior and viability as described, for example, in Reddy *et al.*, *Nature Genetics* **20**:198-202 (1998).

Bacterial toxicity assays are described, for example, in Onodera *et al.*, *FEBS Lett.* **399**:135-9 (1996). Yeast toxicity assays are described, for example, in Krobisch and Linquist, *Proc.*

Natl. Acad Sci. USA **97**:1589-1594 (2000). Toxicity and apoptosis assays in *Caenorhabditis*

10 *elegans* are described, for example, in Faber *et al.*, *Proc. Natl. Acad Sci. USA* **96**:179-184 (1999).

Additional functions include transcriptional activation (direct or indirect through one or more intermediates), transcriptional repression, the ability to bind or interact with proteins *in vitro* or *in vivo*, and the ability to modulate protein folding or transport. Such assays are described further below or are otherwise known in the art. As the proteins affect neural function and neurodegeneration, such biological activities also include behavioral characteristics of the organism. Useful functional assays for characterizing polypeptides and identifying modulators of polyglutamine toxicity therefore also include behavioral assays.

Yet another biological activity of a polypeptide is the ability to bind to an antibody which binds a polypeptide as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. Thus, a modified HDJ1, TPR2 or MLF polypeptide that binds an antibody to which a polypeptide set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 binds has the requisite biological activity. Antibody binding can be tested using a variety of methods known in the art.

25 Thus, in another embodiment, the invention provides functional polypeptides or functional subsequences thereof that share at least 65% identity with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. In other embodiments, the polypeptides have at least 75% identity with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, more likely at least 85% identity with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, or 30 90%, 95%, or more identity with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID

NO:7. The polypeptides of the invention may have one or more of the functions or biological activities described herein.

The invention also provides functional subsequences of HDJ1, TPR2 or MLF polypeptides. As used herein, the term "functional subsequence" refers to a polypeptide fragment that retains at least one function or biological activity characteristic of a full length counterpart polypeptide as described herein. Functional subsequences can therefore vary in size from a polypeptide as small as an epitope capable of binding an antibody molecule (*i.e.*, about five amino acids) up to the entire length of a HDJ1, TPR2 or MLF polypeptide.

Functional HDJ1, TPR2 or MLF subsequences are at least ten amino acid residues in length; more likely, 20 or more amino acid residues in length; and most likely, at least 30, 40, 50 or more amino acid residues in length, *e.g.*, 60, 75, 80, 90, 100, 125, 150, 200, 250, or more.

Particular examples of functional subsequences contain one or more domains that are likely to be important for *in vivo* activity. By inference from the structure of tetratricopeptide proteins, for example, for TPR2, a functional subsequence may include a J domain or one or more of the tetratricopeptide domains, *e.g.*, TPR₁ approximately amino acids 45-82; TPR₂ approximately amino acids 83-116; TPR₃ approximately amino acids 117-150; TPR₄ approximately amino acids 231-264; TPR₅ approximately amino acids 277-310; TPR₆ approximately amino acids 315-348; and TPR₇ approximately amino acids 349-382 of SEQ ID NO:1. The 90 amino acid region of MLF that modulates apoptosis is another example of a particular domain likely to have function.

Functional polypeptides include, for example, conservative substitutions of the amino acid sequences set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. As used herein, the term "conservative substitution" denotes the replacement of an amino acid residue by another, chemically or biologically similar residue. Examples of conservative substitutions include the substitution of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the substitution of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid.

Functional polypeptides further include "chemical derivatives," in which one or more of the amino acids therein has a side chain chemically altered or derivatized. Such

derivatized polypeptides include, for example, amino acids in which free amino groups form amine hydrochlorides, p-toluene sulfonyl groups, carobenzoxy groups; the free carboxy groups form salts, methyl and ethyl esters; free hydroxyl groups that form O-acyl or O-alkyl derivatives as well as naturally occurring amino acid derivatives, for example,
5 4-hydroxyproline, for proline, 5-hydroxylysine for lysine, homoserine for serine, ornithine for lysine etc. Also included are amino acid derivatives that can alter covalent bonding, for example, the disulfide linkage that forms between two cysteine residues that produces a cyclized polypeptide.

The polypeptide modifications may be deliberate, as by site-directed (*e.g.*, PCR
10 based) or random mutagenesis (*e.g.*, EMS) or may be spontaneous or naturally occurring. For example, naturally occurring allelic variants can occur by alternative RNA splicing, polymorphisms, or spontaneous mutations of a nucleic acid encoding HDJ1, TPR2 or MLF polypeptide. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant polypeptide without significantly altering a biological activity.
15 Deletion can lead to the development of a smaller active molecule that could have broader utility. For example, it may be possible to remove amino or carboxy terminal or internal amino acids not required for activity. Alternatively, additions to the sequence may provide an additional or increased functionality.

Invention functional polypeptides and subsequences of HDJ1, TPR2 and MLF
20 include all modifications, amino acid substitutions, additions, deletions, insertions and derivatives set forth herein in respect to full length polypeptides, provided that the subsequence so modified retains at least one function or biological activity of HDJ1, TPR2 or MLF polypeptide. Thus, functional polypeptides and subsequences of HDJ1, TPR2 and MLF can have an amino acid sequence that varies from an amino acid sequence set forth in
25 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

Modified polypeptides are included as long as the modified or otherwise altered polypeptide possesses at least one function or biological activity as described herein (*e.g.*, modulates polyglutamine toxicity, cell degeneration, survival, death, apoptosis, development or viability, behavior, or protein aggregation, folding, transport, degradation, etc.) that is
30 detectable using such a functional assay. Thus, to identify functional polypeptides and subsequences one skilled in the art need only test for the requisite function. For example,

recombinantly modifying the candidate polypeptide (*e.g.*, HDJ1, TPR2 and MLF) by deletion, insertion, or mutation of selected regions and testing whether the modified polypeptide maintains its ability to decrease polyglutamine toxicity. Recombinant modification methods are well established and include, for example, producing successively 5 smaller fragments of the polypeptide by nuclease deletion of a polynucleotide encoding the polypeptide, site-directed mutagenesis of the polynucleotide (using polymerase chain reaction, for example), randomly generated mutations of the polynucleotide, etc.

Loss of toxicity suppressing activity indicates that the modified sequences are important for decreasing toxicity whereas an absence of an effect indicates that the sequences 10 may be modified. A modified polypeptide, such as TPR2 or MLF that retains a function of decreasing polyglutamine toxicity when expressed in *Drosophila* can be assayed for cell death and survival activity, if desired. For example, synthesized or recombinantly produced polypeptides can be introduced into cells in culture to determine their ability to protect against polyglutamine toxicity or apoptosis. *In vitro* and *in vivo* assays to measure protein 15 aggregation, transport, folding and degradation as described herein and also known in the art are applicable in testing function of modified polypeptide. In addition to functional assays described herein for identifying functional polypeptides and subsequences, functional polypeptides and subsequences can be identified as having significant sequence homology, in particular, to other proteins or domains whose function has been characterized, for example, 20 the J domain, the tpr domains, the apoptosis modulating domain of MLF, etc.

HDJ1, TPR2 and MLF polypeptides and functional subsequences can be obtained using standard techniques for protein purification, for example, by chromatography (*e.g.*, ion-exchange, size-exclusion, reverse-phase, immunoaffinity etc.). Other protein purification methods known in the art additionally can be used (see *e.g.*, Deutscher *et al.*, Guide to 25 Protein Purification: Methods in Enzymology, Vol. 182, Academic Press, 1990).

Alternatively, HDJ1, TPR2 and ML polypeptides and subsequences can be obtained using recombinant expression methods as described herein or otherwise known in the art. For example, polynucleotide encoding the protein can be produced, inserted into a vector and transformed into host cells using well known techniques described herein and further known 30 in the art (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y., 1989). Following transformation, protein may be isolated and purified in

accordance with conventional methods. For example, lysate prepared from an expression host (*e.g.*, bacteria) can be purified using HPLC, size-exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. HDJ1, TPR2 and MLF polypeptides and subsequences also can be obtained by chemical synthesis using a peptide synthesizer (*e.g.*, Applied Biosystems, Inc., Foster City, CA; Model 430A or the like).

The invention also provides isolated polynucleotides encoding polypeptides. In one embodiment, an isolated polynucleotide sequence has about 65% or more identity to a *Drosophila* TPR2 (dTPR2) sequence set forth as SEQ. ID NO:2, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 11. In one aspect, the polynucleotide encodes a polypeptide that has a function or biological activity, for example, decreases polyglutamine toxicity. In another aspect, the polynucleotide encodes a subsequence of TPR2 that decreases polyglutamine toxicity. In additional aspects, the polynucleotide encodes a polypeptide that decreases cell death or apoptosis, increases cell survival, proliferation or differentiation, improves development, viability, or behavior, modulates neuron excitability, or decreases protein aggregation (intracellular or extracellular), misfolding, degradation, or aberrant or deficient transport. In yet other aspects, the polynucleotide is operatively linked to an expression control element.

In another embodiment, an isolated polynucleotide sequence has about 65% or more identity to a *Drosophila* MLF (dMLF) sequence set forth as SEQ. ID NO:4, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 12. In one aspect, the polynucleotide encodes a polypeptide that has a function or biological activity, for example, decreases polyglutamine toxicity. In another aspect, the polynucleotide encodes a subsequence of MLF that decreases polyglutamine toxicity. In additional aspects, the polynucleotide encodes a polypeptide that decreases cell death or apoptosis, aberrant development or behavior, increases cell survival, proliferation, differentiation, or viability, or decreases protein aggregation (intracellular or extracellular), misfolding, degradation, or aberrant or deficient transport. In yet other aspects, the polynucleotide is operatively linked to an expression control element.

The TPR2 gene corresponds to a cDNA of 2239 nucleotides. The MLF gene corresponds to a cDNA of 1753 nucleotides. Specifically disclosed herein are nucleic acid

sequences for *Drosophila* TPR2 and MLF (SEQ ID NO:2 and SEQ ID NO:4, respectively; Figures 9 and 10)

As used herein, the terms “polynucleotide” and “nucleic acid” are used interchangeably to refer to all forms of nucleic acid, oligonucleotides, primers, and probes, 5 including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Polynucleotides include genomic DNA, cDNA and antisense DNA, and spliced or unspliced mRNA, rRNA tRNA and antisense RNA (*e.g.*, RNAi). Polynucleotides include naturally occurring, synthetic, and intentionally altered or modified polynucleotides as well as analogues and derivatives. Alterations can result in increased stability due to resistance to nuclease 10 digestion, for example. Polynucleotides can be double, single or triplex, linear or circular, and can be of any length.

The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Degenerate sequences may not selectively hybridize to other invention 15 nucleic acids; however, they are nonetheless included as they encode invention HDJ1, TPR2 and MLF polypeptides and functional subsequences thereof. Thus, in another embodiment, degenerate nucleotide sequences that encode HDJ1, TPR2 and MLF polypeptides set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7, and functional subsequences thereof, are provided.

20 The polynucleotide sequences for HDJ1, TPR2 and MLF include complementary sequences (*e.g.*, antisense to all or a part of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8). Antisense polynucleotides, to decrease activity or expression of HDJ1, TPR2 and MLF, for example, do not require expression control elements to function *in vivo*. However, antisense may be encoded by a nucleic acid and such a nucleic acid may be 25 operatively linked to an expression control element for sustained or increased expression of the encoded antisense in cells or *in vivo*. Sequences encoding dominant negative forms of HDJ1, TPR2 and MLF also are included. Such dominant negative forms may inhibit interaction of the native endogenous protein with a signaling pathway thereby modulating the pathway.

30 Further included are double stranded RNA sequences from a HDJ1, TPR2 and MLF coding region. The use of double stranded RNA sequences (known as “RNAi”) for

inhibiting gene expression, for example, in insects and in other organisms is known in the art (Kennerdell *et al.*, *Cell* **95**:1017-1026 (1998); Fire *et al.*, *Nature*, **391**:806-811 (1998)). Such sequences can interfere with HDJ1, TPR2 and MLF activity or expression and be useful for increasing polyglutamine toxicity or sensitivity to polyglutamine toxicity, decreasing cell 5 survival, increasing apoptosis, etc. An effective amount of double stranded RNA from the coding region of HDJ1, TPR2 or MLF, HDJ1, TPR2 and MLF antisense polynucleotides and polynucleotides encoding dominant negative forms of HDJ1, TPR2 and MLF can inhibit HDJ1, TPR2 and MLF function or expression and are therefore useful in the therapeutic and other methods of treating aberrant or undesirable cell survival, proliferation (*e.g.*, cancer) or 10 differentiation, as described herein. Such invention polynucleotides can be further contained within carriers or vectors suitable for passing through a cell membrane for cytoplasmic delivery, and can be modified so as to be nuclease resistant in order to enhance their stability or efficacy in the invention methods and compositions, for example.

Thus, in another embodiment, polynucleotides encoding HDJ1, TPR2 and MLF 15 including the nucleotide sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8, as well as nucleic acid sequences complementary to the sequence (*e.g.*, antisense polynucleotides) are provided. When a polynucleotide sequence is RNA, the deoxyribonucleotides A, G, C, and T of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8 are replaced by ribonucleotides A, G, C, and U, respectively.

It is understood that HDJ1, TPR2 and MLF homologs, including HDJ1, TPR2 and 20 MLF homologs having polymorphisms as set forth herein, also are included and are useful in practicing the methods of the invention. Nucleic acid probes based on SEQ ID NO:2 and SEQ ID NO:4 can be used to identify such homologs, for example. Homologs are envisioned to be present in living organisms that reproduce sexually including animals, such 25 as mammals.

As used herein, the term "polymorphism" refers to a naturally occurring or 30 synthetically produced (*e.g.*, EMS induced mutagenesis) nucleotide sequence difference that may or may not encode an altered amino acid sequence. Thus, polymorphisms can be silent such that a function or biological activity generally is comparable to unaltered polypeptide, or be detectable. For example, a polymorphism may inhibit or enhance/activate a HDJ1,

TPR2 and MLF polypeptide function or biological activity (*e.g.*, increase or decrease its suppression of polyglutamine toxicity).

Polynucleotides encoding portions of HDJ1, TPR2 and MLF polypeptide are included herein. Particular examples are nucleic acid sequences that encode HDJ1, TPR2 and MLF functional subsequences. As used herein, the term "functional polynucleotide" denotes a polynucleotide that encodes a functional polypeptide as described herein. Thus, the invention includes polynucleotides encoding a polypeptide having a function or biological activity of an amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7. Moreover, as polynucleotides having nonsense (stop) mutations in a nucleic acid sequence can still encode a functional subsequence of HDJ1, TPR2 and MLF polypeptides, such polynucleotides also are included.

Additional polynucleotides included are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is of sufficient length to permit a selective hybridization to a TPR2 and MLF nucleic acid set forth in SEQ ID NO:2 and SEQ ID NO:4, and a nucleic acid encoding an amino acid sequence set forth in SEQ ID NO:1 and 3 or functional subsequences thereof, provided that the polynucleotide fragments are distinct from the ESTs set forth in Figures 11 or 12. Thus, in another embodiment, fragments of SEQ ID NO:2 and SEQ ID NO:4; SEQ ID NO:2 and SEQ ID NO:4, where T can also be U; nucleic acid sequences complementary to SEQ ID NO:2 and SEQ ID NO:4 that are at least 15 bases in length; and nucleic acid sequences that selectively hybridize to DNA that encodes TPR2 and MLF polypeptide set forth in SEQ ID NO:1 and SEQ ID NO:3, respectively, also are provided.

Polynucleotide fragments of at least 15 bases in length can be used to screen for TPR2 and MLF related genes in other organisms, such as mammals or insects, and are referred to herein as "probes." Invention probes additionally can have a "label" or "detectable moiety" linked thereto that provides a detection signal (*e.g.*, radionuclides, fluorescent, chemi- or other luminescent moieties). If necessary, additional reagents can be used in combination with the detectable moieties to provide or enhance the detection signal. Such labels and detectable moieties also can be linked to invention TPR2 and MLF polypeptides, functional fragments, antibodies, and the compounds that modulate a

polyglutamine toxicity or expression of a polynucleotide encoding TPR2 and MLF polypeptide disclosed herein.

Polynucleotide fragments also are useful for diagnostic purposes as under or aberrant expression or activity of TPR2 or MLF is likely to be associated with or contribute to 5 polyglutamine toxicity, or protein aggregative, neurodegenerative or musculardegenerative disorders, prion diseases, or proliferative, developmental, viability, or behavioral disorders, etc. as set forth herein. Such polynucleotide fragments also are useful for detecting the presence or amount of a TPR2 or MLF transgene in a transgenic animal.

Thus, in accordance with the present invention, there are provided isolated 10 polynucleotides that selectively hybridize to the polynucleotides described herein. In one embodiment, an isolated polynucleotide sequence hybridizes under stringent conditions to a *Drosophila* TPR2 (dTPR2) sequence set forth as SEQ. ID NO:2, with the proviso that the polynucleotide sequence is distinct from the EST sequences set forth in Figure 11. In one aspect, the polynucleotide sequence comprises a polynucleotide having 20 or more 15 contiguous nucleotides. In another aspect, the polynucleotide sequence comprises a polynucleotide having 50 or more contiguous nucleotides. In various additional aspects, the polynucleotide sequence comprises a polynucleotide having 60 or more, 70 or more, 80 or more, 100 or more, 120 or more, 140 or more, 160 or more contiguous nucleotides, up to the full length sequence.

20 In another embodiment, an isolated polynucleotide sequence hybridizes under stringent conditions to a *Drosophila* MLF (dMLF) sequence set forth as SEQ. ID NO:4, with the proviso that the polynucleotide sequence is distinct from the EST sequences set forth in Figure 12. In one aspect, the polynucleotide sequence comprises a polynucleotide having 20 or more contiguous nucleotides. In another aspect, the polynucleotide sequence comprises a 25 polynucleotide having 50 or more contiguous nucleotides. In various additional aspects, the polynucleotide sequence comprises a polynucleotide having 60 or more, 70 or more, 80 or more, 100 or more, 120 or more, 140 or more, 160 or more contiguous nucleotides, up to the full length sequence.

Hybridization refers to the binding between complementary nucleic acid sequences 30 (e.g., sense/antisense). As used herein, the term “selective hybridization” refers to hybridization under moderately stringent or highly stringent conditions, which can

distinguish TPR2 and MLF related nucleotide sequences from unrelated sequences (see *e.g.*, the hybridization techniques described in Sambrook *et al.*, 1989, *supra*). Screening procedures which rely on hybridization allow isolation of related nucleic acid sequences, such as a TPR2 and MLF homologs, orthologues, polymorphic sequences, etc. (*e.g.*, cDNA or genomic DNA), from any organism.

In nucleic acid hybridization reactions, the conditions used in order to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of sequence complementarity, sequence composition (*e.g.*, the GC v. AT content), and type (*e.g.*, RNA v. DNA) of the hybridizing regions can be considered in selecting particular hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

As is understood by those skilled in the art, the Tm (melting temperature) refers to the temperature at which the binding between two sequences is no longer stable. For two sequences to form a stable hybrid, the temperature of the reaction must be less than the Tm for the particular hybridization conditions. In general, the stability of a nucleic acid hybrid decreases as the sodium ion decreases and the temperature of the hybridization reaction increases.

Typically, wash conditions are adjusted so as to attain the desired degree of stringency. Thus, hybridization stringency can be determined, for example, by washing at a particular condition, *e.g.*, at low stringency conditions or high stringency conditions, or by using each of the conditions, *e.g.*, for 10-15 minutes each, in the order listed below, repeating any or all of the steps listed. Optimal conditions for selective hybridization will vary depending on the particular hybridization reaction involved.

An example of a moderately stringent hybridization condition is as follows: 2 x SSC/0.1% SDS at about 37°C or 42°C (hybridization conditions); 0.5 x SSC/0.1% SDS at about room temperature (low stringency wash); 0.5 x SSC/0.1% SDS at about 42°C (moderate stringency wash). An example of a moderately-high stringent hybridization condition is as follows: 2 x SSC/0.1% SDS at about 37°C or 42°C (hybridization conditions); 0.5 x SSC/0.1% SDS at about room temperature (low stringency wash); 0.5 x SSC/0.1% SDS at about 42°C (moderate stringency wash); and 0.1 x SSC/0.1% SDS at about 52°C (moderately-high stringency wash). An example of high stringency hybridization

conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.5 x SSC/0.1% SDS at about room temperature (low stringency wash); 0.5 x SSC/0.1% SDS at about 42°C (moderate stringency wash); and 0.1 x SSC/0.1% SDS at about 65°C (high stringency wash).

5 Homologs of HDJ1, TPR2 and MLF can be identified by sequence similarity. *i.e.*, at least 50% sequence identity between nucleotide sequences, likely at least 60% sequence identity between nucleotide sequences, more likely at least 75% sequence identity between nucleotide sequences and most likely at least 80% sequence identity between nucleotide sequences. Highly homologous sequences will have at least 85%, 90%, 95% or more 10 sequence identity. Sequence homology is calculated based on a reference sequence, which may be a region of a larger sequence, such as a conserved motif, coding region, flanking region, etc.

A reference sequence will usually be at least 18 nucleotides long, more usually at least 30 nucleotides long, and may extend to the complete sequence that is being compared.

15 The extent of sequence identity between two sequences can be ascertained using various computer programs and mathematical algorithms known in the art. Such algorithms that calculate percent sequence identity (homology) generally account for sequence gaps and mismatches over the region of similarity. For example, a BLAST (*e.g.*, BLAST 2.0) search algorithm (see, *e.g.*, Altschul *et al.* *J. Mol. Biol.* **215**:403-10 (1990), which is publicly 20 available through NCBI at <http://www.ncbi.nlm.nih.gov>) has exemplary search parameters as follows: Mismatch -2; gap open 5; gap extension 2. For polypeptide sequence comparisons, MacVector PPC 6.0.1 software program parameters for *Drosophila* dTPR2 and human TPR2 were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 10.0: Extend gap penalty 0.1; similarity matrix blosum. For *Drosophila* dMLF and human MLF the program 25 parameters were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 1.0: Extend gap penalty 0.1; similarity matrix blosum. EST search parameters were BLASTN 2.0a19MP.

Thus, in one embodiment, a polynucleotide sequence of the invention comprises a 30 sequence having 65% or more homology to a sequence set forth in SEQ ID NO:2, as determined using a BLAST search algorithm, provided that the polynucleotide sequence is distinct from the EST sequences set forth in Figure 11. In another embodiment, a

polynucleotide sequence of the invention comprises a sequence having 65% or more homology to a sequence set forth in SEQ ID NO:4, as determined using a BLAST search algorithm, provided that the polynucleotide sequence is distinct from the EST sequences set forth in Figure 12. In various additional embodiments, a polynucleotide sequence of the invention can have at least 70%, 75%, 80%, 90%, or 95% sequence identity to a sequence set forth in SEQ ID NO:2 or SEQ ID NO:4.

Polynucleotides of the invention can be obtained using various standard cloning and chemical synthesis techniques. Purity of polynucleotides can be determined through sequencing, gel electrophoresis and the like. For example, nucleic acids can be isolated using hybridization as set forth herein or computer-based database screening techniques known in the art. Such techniques include, but are not limited to: (1) hybridization of genomic DNA or cDNA libraries with probes to detect homologous nucleotide sequences; (2) antibody screening to detect polypeptides having shared structural features, for example, using an expression library; (3) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to a nucleic acid sequence of interest; (4) computer searches of sequence databases for related sequences; and (5) differential screening of a subtracted nucleic acid library.

Particular examples of such polynucleotide sequences having high homology to the sequences described herein are polymorphic sequences. Alterations in the sequence include but are not limited to intragenic mutations (*e.g.*, point mutation, splice site and frameshift) and heterozygous or homozygous deletions. Termination signals or mutations that produce a stop codon leading to a terminated translation product may or may not retain a function or biological activity *in vivo* depending on the length of the terminated product, product stability, etc. Detection of sequences having altered nucleotides can be determined by standard methods known to those of skill in the art which include, for example, sequence analysis, Southern blot analysis, PCR based analyses (*e.g.*, multiplex PCR, sequence tagged sites (STSs) and *in situ* hybridization).

Nucleotide probes, which correspond to a part of a TPR2 or MLF sequence encoding the protein, can be based upon TPR2 and MLF sequence, such as that set forth in SEQ ID NO:2 and SEQ ID NO:4, respectively. Alternatively, oligopeptide stretches of an amino acid sequence can be used to deduce the nucleic acid sequence based on the genetic code;

however, as code degeneracy must be taken into account, a mixed addition reaction of a degenerate probe population can be performed. For such screening, hybridization is preferably performed on either single-stranded or denatured double-stranded nucleic acid. Alternatively, where at least two stretches of amino acid sequence of a polypeptide is known,
5 polymerase chain reaction (PCR) of genomic DNA or cDNA using a mixed population of degenerate probes deduced from the two stretches of amino acid sequence, can be used to amplify a related polynucleotide sequence for subsequent cloning and characterization.

Another alternative for identifying similar or homologous nucleic acid sequences is to screen expressed DNA sequences. For example, among standard procedures for isolating
10 DNA sequences of interest is by the formation of plasmid or phage-libraries. Thus, cDNA can be derived from reverse transcription of mRNA present in donor cells and cloned into an appropriate expression phage or plasmid. When used in combination with polymerase chain reaction (PCR) technology, rare expression products can be cloned and expressed. Lambda gt11 is one particular example of a phage suitable for expressing a cDNA encoding
15 polypeptides or peptides having similar epitopes as HDJ1, TPR2 or MLF. Antibodies can be used to detect an expression product indicative of the presence of the corresponding cDNA, for example. As various types of libraries from a variety of different animals and cells are commercially available or can be produced from donor cells, tissue or whole organisms using well known methods, expression screening affords the capability of identifying homologs to
20 HDJ1, TPR2 and MLF polypeptides from a variety of other sources.

An alteration in a TPR2 and MLF coding sequence can be, but is not limited to, a point mutation, nonsense mutation, missense mutation, splice site mutation, or a frameshift mutation. The alteration also can be a deletion of a segment of a nucleic acid encoding a TPR2 and MLF polypeptide such that a biological activity or function of the TPR2 and MLF
25 polypeptide is removed or eliminated. Alternatively, an alteration can allow for expanded (e.g., in tissues/cells that do not normally express TPR2 and MLF) or for increased expression, for example, through the inactivation or deletion of an expression silencer.

An alteration in a TPR2 and MLF non-coding nucleic acid sequence (*i.e.*, 5' and 3' non-coding flanking sequences and introns of a genomic sequence) can be, for example, a
30 point mutation or deletion. A point mutation or deletion of a transcriptional control element conferring TPR2 and MLF expression can inhibit or eliminate TPR2 and MLF expression

thereby increasing polyglutamine toxicity in an organism, for example. Another non-limiting example of an alteration is a deletion of a 3' flanking sequence that confers RNA stability. Point mutation or deletion of an intronic splice site is an additional example of a disrupted TPR2 and MLF gene. It is understood that alterations which disrupt TPR2 and MLF genes 5 can be present simultaneously in coding and non-coding regions of a TPR2 and MLF nucleic acid sequence.

Another non-limiting example of a disrupted gene is a nucleic acid encoding a polypeptide into which another nucleic acid sequence has been inserted. An endogenous nucleic acid having such an insertion can eliminate expression of the endogenous gene.

10 dHDJ1, dTPR2 and dMLF polypeptides set forth as SEQ ID NO:1 and SEQ ID NO:3 when introduced into *Drosophila*, decrease polyglutamine toxicity. The mammalian homologues of these genes share structural features that likely account for this activity. Thus, invention dHDJ1, dTPR2 and dMLF polynucleotides and encoded polypeptides and functional subsequences, and the HDJ1, TPR2 and MLF mammalian homologues (e.g., SEQ 15 ID NO:5 and SEQ ID NO:7), are useful in treating polyglutamine toxicity and related disorders in human subjects, as described herein.

Accordingly, the invention provides polynucleotides including an expression control element controlling expression of an operatively linked HDJ1, TPR2 or MLF nucleic acid. In one embodiment, the nucleic acid encodes a sequence set forth in SEQ ID NO:1, SEQ ID 20 NO:3, SEQ ID NO:5 or SEQ ID NO:7. In another embodiment, the nucleic acid encodes a functional subsequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. In one aspect, a functional subsequence comprises a J domain (e.g., TPR2 amino acids 401 to 469). Such polynucleotides containing an expression control element controlling expression of a nucleic acid can be modified or altered as set forth herein, so long as the modified or 25 altered polynucleotide has one or more functions or biological activities.

For expression in cells, invention polynucleotides, if desired, may be inserted into a vector. Accordingly, invention compositions and methods further include polynucleotide sequences inserted into a vector.

The term "vector" refers to a plasmid, virus or other vehicle known in the art that can 30 be manipulated by insertion or incorporation of a polynucleotide. Such vectors can be used for genetic manipulation (*i.e.*, "cloning vectors") or can be used to transcribe or translate the

inserted polynucleotide (*i.e.*, "expression vectors"). A vector generally contains at least an origin of replication for propagation in a cell and a promoter. Control elements, including expression control elements as set forth herein, present within a vector are included to facilitate proper transcription and translation (*e.g.*, splicing signal for introns, maintenance of 5 the correct reading frame of the gene to permit in-frame translation of mRNA and, stop codons etc.).

By "promoter" is meant a minimal sequence sufficient to direct transcription. Although generally located 5' of the coding sequence, they can be located in introns or 3' of the coding sequence. Both constitutive and inducible promoters are included in the invention 10 (see *e.g.*, Bitter *et al.*, *Methods in Enzymology*, 153:516-544 (1987)). Inducible promoters are activated by external signals or agents. Repressible promoters are inactivated by external signals or agents. Derepressible promoters are normally inactive in the presence of an external signal but are activated by removal of the external signal or agent. As discussed, also included are promoter elements sufficient to render gene expression controllable for 15 specific cell-types, tissues or physiological conditions (*e.g.*, heat shock, glucose starvation).

When cloning in bacterial systems, constitutive promoters such as T7 and the like, as well as inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) may be used. In yeast, a number of vectors containing constitutive or inducible promoters may be used (see *e.g.*, Current Protocols in Molecular Biology, 2:13 (1988); Grant 20 *et al.*, *Methods in Enzymology*, 153:516-544 (1987); Glover, *DNA Cloning*, II:3 (1986); Bitter, *Methods in Enzymology*, 152:673-684 (1987); and The Molecular Biology of the Yeast Saccharomyces, Eds. Strathern *et al.*, Cold Spring Harbor Press, Vols. I and II (1982). A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (Rothstein, DNA Cloning, A Practical Approach, 11:3 (1986)). Alternatively, 25 vectors that facilitate integration of foreign nucleic acid sequences into a yeast chromosome, via homologous recombination, for example, are known in the art and can be used. Yeast artificial chromosomes (YAC) are typically used when the inserted polynucleotides are too large for more conventional yeast expression vectors (*e.g.*, greater than about 12 kb).

When cloning in mammalian cell systems, constitutive promoters such as SV40, RSV 30 and the like or inducible promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the mouse mammary tumor

virus long terminal repeat; the adenovirus late promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences of the invention. Mammalian expression systems that utilize recombinant viruses or viral elements to direct expression may be engineered, if desired. For example, when using adenovirus expression vectors, the coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. Alternatively, the vaccinia virus 7.5K promoter may be used (see *e.g.*, Mackett *et al.*, *Proc. Natl. Acad. Sci. USA*, **79**:7415-7419 (1982); Mackett *et al.*, *J. Virol.*, **49**:857-864 (1984); and Panicali *et al.*, *Proc. Natl. Acad. Sci. USA*, **79**:4927-4931 (1982)).

Vectors based on bovine papilloma virus (BPV) have the ability to replicate as extrachromosomal elements (Sarver *et al.*, *Mol. Cell. Biol.*, **1**:486 (1981)). Shortly after entry of an extrachromosomal vector into mouse cells, the vector replicates to about 100 to 200 copies per cell. Because transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, a high level of expression occurs. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as the *neo* gene, for example. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the gene in host cells (Cone *et al.*, *Proc. Natl. Acad. Sci. USA*, **81**:6349-6353 (1984)). High-level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionein IIA promoter and heat shock promoters.

Mammalian expression systems further include vectors specifically designed for *in vivo* applications. Such systems include adenoviral vectors (U.S. Patent Nos. 5,700,470 and 5,731,172), adeno-associated vectors (U.S. Patent Nos. 5,354,678, 5,604,090, 5,780,447), herpes simplex virus vectors (U.S. Patent No. 5,501,979) and retroviral vectors (U.S. Patent Nos. 5,624,820, 5,693,508 and 5,674,703 and WIPO publications WO92/05266 and WO92/14829). Bovine papilloma virus (BPV) has also been employed in gene therapy (U.S. Patent No. 5,719,054). Such vectors also include CMV based vectors (U.S. Patent No. 5,561,063). For targeting dividing neurons *in vivo*, genetic material and a growth factor may be administered for *in vivo* expression (U.S. Patent No. 6,071,889). For targeting post-mitotic neurons *in vivo* (*e.g.*, sympathetic, dopaminergic, or cortical), adenovirus vectors containing the nucleic acid can be administered for *in vivo* expression (U.S. Patent

No. 6,060,247). For targeting muscle *in vivo*, myoblasts can be transformed *ex vivo* and reintroduced into muscle tissue of a subject (U.S. Patent No. 5,538,722). In addition to viral vectors suitable for expression *in vivo*, lipids for intracellular delivery of polypeptides (including antibodies) and polynucleotides also are contemplated (U.S. Patent 5 Nos. 5,459,127 and 5,827,703). Combinations of lipids and adeno-associated viral material also can be used for *in vivo* delivery (U.S. Patent No. 5,834,441).

In accordance with the present invention, polynucleotide sequences encoding HDJ1, TPR2 and MLF polypeptide or functional subsequences may be inserted into an expression vector for expression *in vitro* (e.g., using *in vitro* transcription/translation kits, which are available commercially), or may be inserted into an expression vector that contains a promoter sequence which facilitates transcription in either prokaryotes or eukaryotes (e.g., an insect cell) by transfer of an appropriate nucleic acid into a suitable cell. A cell into which a vector can be propagated and its nucleic acid transcribed, or encoded polypeptide expressed, is referred to herein as a "host cell." The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. For example, although some progeny may contain mutations in the introduced vector, such progeny are nevertheless included when the term "host cell" is used.

Host cells include but are not limited to microorganisms such as bacteria, yeast, plant, insect and mammalian organisms. For example, bacteria transformed with recombinant bacteriophage nucleic acid, plasmid nucleic acid or cosmid nucleic acid expression vectors containing a HDJ1, TPR2 and MLF coding sequence; yeast transformed with recombinant expression vectors containing a HDJ1, TPR2 and MLF coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing a HDJ1, TPR2 and MLF coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing a HDJ1, TPR2 and MLF coding sequence; or animal cell systems infected with recombinant virus expression vectors (e.g., retroviruses, adenovirus, vaccinia virus) containing a HDJ1, TPR2 and MLF coding sequence, or transformed animal cell systems engineered for stable expression. .

For long-term expression in host cells, expression vectors that contain viral origins of replication, for example, can be transformed. Although not wishing to be bound or so limited by any particular theory, stable maintenance of expression vectors in mammalian cells is believed to occur by integration of the vector into a chromosome of the host cell. Optionally,
5 the expression vector also can contain a nucleic acid encoding a selectable or identifiable marker conferring resistance to a selective pressure thereby allowing cells having the vector to be identified, grown and expanded. Alternatively, the selectable marker can be on a second vector that is cotransfected into a host cell with a first vector containing an invention polynucleotide.

10 A number of selection systems may be used to identify or select for transformed host cells, including, but not limited to the herpes simplex virus thymidine kinase gene (Wigler *et al.*, *Cell*, **11**:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase gene (Szybalska *et al.*, *Proc. Natl. Acad. Sci. USA*, **48**:2026 (1962)), and the adenine phosphoribosyltransferase (Lowy *et al.*, *Cell*, **22**:817 (1980)) genes can be employed in tk-,
15 hgprt- or aprt- cells respectively. Additionally, antimetabolite resistance can be used as the basis of selection for *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. USA*, **77**:3567 (1980); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA*, **78**:1527 (1981)); the *gpt* gene, which confers resistance to mycophenolic acid (Mulligan *et al.*, *Proc. Natl. Acad. Sci. USA*, **78**:2072 (1981); the *neomycin* gene, which confers resistance to the
20 aminoglycoside G-418 (Colberre-Garapin *et al.*, *J. Mol. Biol.*, **150**:1 (1981)); and the *hygromycin* gene, which confers resistance to hygromycin (Santerre *et al.*, *Gene*, **30**:147 (1984)).

As used herein, the term "transformation" means a genetic change in a cell following incorporation of nucleic acid or polypeptide exogenous to the cell. Thus, a "transformed cell" is a cell into which (or a progeny of which) a nucleic acid or polypeptide molecule has been introduced by means of recombinant DNA techniques.
25

Transformation of a host cell with DNA may be carried out by conventional techniques known to those skilled in the art. For example, when the host cell is a eukaryote, methods of DNA transformation include, for example, calcium phosphate co-precipitates,
30 conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, and viral vectors. Eukaryotic cells also can be cotransformed

with DNA sequences with or without a selectable marker. Particularly useful eukaryotic host cells are cell lines in which polyglutamine toxicity can be assayed *in vitro*, or cell lines related to or obtained from *in vivo* tissues that have or can develop polyglutamine toxicity *in vivo*. When the host is prokaryotic (*e.g.*, *E. coli*), competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl₂ method using procedures well known in the art. Transformation of prokaryotes also can be performed by protoplast fusion of the host cell.

Host cells also are useful in the various screening methods described herein. For example, compounds or trans-activating protein factors that induce or stimulate expression of a target gene can be screened for by transforming host cells with a promoter or regulatory region of the target gene operatively linked to a reporter construct. Candidate target gene promoters and regulatory regions include, for example, dHDJ1, dTPR2 and dMLF, and their mammalian (*e.g.*, human) homologues hHDJ1, hTPR2 and hMLF.

Reporters such as a cDNA for green fluorescence protein (GFP), or others that directly or indirectly provide a signal (*e.g.*, light) can be located 3' of the promoter. Since it would be advantageous to be able to screen a large number of compounds, to facilitate and accelerate the screening process, the sequence encoding a protein secretion signal, functional in the cell type used, is fused in-frame with the coding sequences for GFP (see, for example, Figure 15). Increased expression of secreted GFP, or other suitable reporter, is used to identify compounds that may have a prophylactic or therapeutic value due to their ability to increase expression of the target gene. Transformed cell lines (*e.g.*, neuron, retinal, muscle or mesoderm) can be cultured in one or more 96 well (or more) plates for large-scale screening, and various compounds and doses may be added to each of the wells. If a compound increases promoter activity, GFP is expressed in the cell and secreted into the culture medium. To detect fluorescence, appropriate wavelength of ultraviolet light is shone on each well of the plate in a plate reader and all plates are analyzed efficiently for compounds that increase promoter activity. Such compounds and transactivating factors are suitable candidates for use in the methods described herein.

Accordingly, in another embodiment, methods of identifying compounds and transactivating factors that modulate expression of genes that modulate polyglutamine toxicity are provided. In one embodiment, a method includes contacting an expression

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control element (e.g., promoter or other regulatory region) of such a gene with a test compound, and assaying for increased or decreased activity of an operatively linked reporter. In one aspect, a regulatory region comprises a polynucleotide sequence located 5' of a coding sequence for dHDJ1, dTPR2 or dMLF, as set forth in SEQ ID NO:9, SEQ ID NO:10 and 5 SEQ ID NO:11, respectively (see, for example, Figures 16 to 18). In additional aspects, a regulatory region comprises a portion of a polynucleotide sequence located 5' of a coding sequence for dHDJ1, dTPR2 or dMLF, as set forth in SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11, wherein the sequence includes a polynucleotide sequence located 100 base-pairs, 250 base-pairs, 0.5Kb, 1.0Kb, 2.0Kb, 3.0Kb, 4.0Kb, 5.0 Kb or more 5' of the ATG 10 start site of the coding sequence.

HDJ1, TPR2 and MLF polypeptides and functional subsequences can be used to generate additional reagents, such as antibodies. Thus, in accordance with the present invention, antibodies that bind to a dTPR2 and dMLF polypeptide, functional subsequences or to antigenic fragments thereof are provided. Antibody comprising polyclonal antibodies, 15 pooled monoclonal antibodies with different epitopic specificities, and distinct monoclonal antibody preparations, also are provided. Such antibodies include those that distinguish dTPR2 and dMLF from their human homologues.

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The term "antibody" includes intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding to an epitopic determinant present in a dTPR2 or dMLF polypeptide subsequence thereof. Other antibody fragments are included so long as the fragment retains the ability to selectively bind with its antigen.

5 Antibodies that bind to dTPR2 and dMLF polypeptides can be prepared using intact polypeptide or small peptide fragments thereof as the immunizing antigen. For example, as it may be desirable to produce antibodies that specifically bind to the amino- or carboxy-terminal domains or functional subsequences of dTPR2 and dMLF, amino- carboxy-terminal and functional subsequence fragments of dTPR2 and dMLF can be used as the immunizing 10 antigen. The polypeptide or peptide used to immunize an animal which is derived from translated DNA or chemically synthesized can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the immunizing peptide include, for example, keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid.

15 Monoclonal antibodies are made by methods well known to those skilled in the art and are also provided (Kohler *et al.*, *Nature*, **256**:495 (1975); and Harlow *et al.*, "Antibodies: A Laboratory Manual", p 726, Cold Spring Harbor Pub. (1988)). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by analyzing a serum sample, removing the 20 spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques which include, for example, affinity chromatography with Protein-A Sepharose, 25 size-exclusion chromatography, and ion-exchange chromatography (see e.g., Coligan *et al.*, Current Protocols in Immunology sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; and Barnes *et al.*, "Methods in Molecular Biology," **10**:79-104, Humana Press (1992)).

The preparation of polyclonal antibodies is well-known to those skilled in the art (see, e.g., Green *et al.*, Immunochemical Protocols, pp 1-5, Manson, ed., Humana Press (1992); 30 Harlow *et al.* (1988), *supra*; and Coligan *et al.* (1992), *supra*, section 2.4.1). Those of skill in the art will know of various techniques common in the immunology arts for purification

and/or concentration of polyclonal and monoclonal antibodies (see e.g., Coligan *et al.*, Unit 9, "Current Protocols in Immunology," Wiley Interscience (1994)).

Antibodies of the invention also can be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465, 1991, and Losman *et al.*, *Int. J. Cancer*, **46**:310 (1990). Alternatively, a useful anti-dTPR2 or dMLF antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts.

The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Natl. Acad. Sci. USA*, **86**:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature*, **321**:522 (1986); Riechmann *et al.*, *Nature*, **332**:323 (1988); Verhoeyen *et al.*, *Science*, **239**:1534 (1988); Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, **89**:4285 (1992); Sandhu, *Crit. Rev. Biotech.*, **12**:437 (1992); and Singer *et al.*, *J. Immunol.*, **150**:2844 (1993).

Antibodies of the invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library (see e.g., Barbas *et al.*, *Methods: A Companion to Methods in Enzymology*, **2**:119 (1991); Winter *et al.*, *Ann. Rev. Immunol.*, **12**:433 (1994)). Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens and can be used to produce human antibody-secreting

hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.*, 7:13 (1994); Lonberg *et al.*, *Nature*, 368:856 (1994); and Taylor *et al.*, *Int. Immunol.*, 6:579 (1994).

Antibody fragments (*e.g.*, Fab, F(ab')₂, and Fv) of the present invention can be prepared by proteolytic hydrolysis of the antibody, for example, by pepsin or papain digestion of whole antibodies. In particular, antibody fragments produced by enzymatic cleavage with pepsin provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Patent Nos. 4,036,945 and 4,331,647, and references contained therein, (see also Nisonhoff *et al.*, *Arch. Biochem. Biophys.*, 89:230 (1960); Porter, *Biochem. J.*, 73:119 (1959); Edelman *et al.*, *Methods in Enzymology*, 1:422 (1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4, *supra*). Alternatively, antibody fragments can be prepared by expression of a nucleic acid encoding an antibody fragment in *E. coli*, for example.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar *et al.* (*Proc. Natl. Acad. Sci. USA*, 69:2659 (1972)). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (*e.g.*, Sandhu, 1992, *supra*). Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising nucleic acid sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow

et al., *Methods: A Companion to Methods in Enzymology* 2:97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271-77 (1993); and Sandhu (1992), *supra*.

Antibodies of the invention are useful for a variety of purposes including, for example, detecting an amount of HDJ1, TPR2 or MLF in a cell or tissue of a subject. Such methods comprise contacting a sample suspected of containing an invention polypeptide (*in vitro* or *in vivo*; in a cell or organism) with an antibody under conditions that allow binding and, detecting the presence of the antibody bound to the query polypeptide thereby detecting the presence of the polypeptide. Such methods are useful for determining the amount of polypeptide produced in the transgenic animals, screening or therapeutic methods of the invention, for example. The presence of the polypeptide can be detected by methods well known in the art, for example, ELISA, immunohistochemical staining, flow cytometry, immunoprecipitation, etc.

Antibodies of the invention also are useful for purifying HDJ1, TPR2 and MLF polypeptides, functional subsequences and antigenic fragments thereof using standard immunopurification techniques known in the art.

Invention antibodies also are contemplated for use in detection assays for diagnostic purposes or for modulating a function or biological activity of a HDJ1, TPR2 and MLF polypeptide or functional subsequence. For example, an antibody that binds a MLF epitope at or near a region that confers a MLF polyglutamine decreasing toxicity can be used to modulate toxicity. An antibody or antibody fragment that binds to a polypeptide can therefore function as an antagonist or, alternatively, can function as an agonist if the antibody or antibody fragment mimics an activator that stimulates or enhances MLF activity.

Invention antibodies that modulate an activity or function of a HDJ1, TPR2 and MLF polypeptide or subsequence are further contemplated as pharmaceutical compositions as described herein. A similar approach may be used with polypeptide fragments of HDJ1, TPR2 or MLF (e.g., dominant negative or agonistic forms) to inhibit or promote interactions with molecules that participate in the cell signaling pathways that modulate polyglutamine toxicity and related conditions.

The invention further provides methods for identifying genes, compounds and transactivating factors that modulate a function or biological activity of the genes that

modulate polyglutamine toxicity. In one embodiment, a method of the invention includes breeding a first animal that exhibits modulated polyglutamine sequence toxicity due to expression or activity of a modulating genetic element, to a second animal having a marker sequence; screening progeny for increased or decreased polyglutamine toxicity; and

5 identifying one or more genes in the progeny animal that modulates function or activity the genetic element that modulates polyglutamine toxicity.

In another embodiment, a method of the invention includes incubating components containing HDJ1, TPR2 and MLF polypeptide or subsequence thereof, or a cell or animal expressing HDJ1, TPR2 and MLF polypeptide or subsequence thereof, and a test compound,

10 under conditions sufficient to allow the components to interact and, determining the effect of the test compound on HDJ1, TPR2 and MLF polypeptide activity or expression (*e.g.*, polyglutamine toxicity).

In cells, proteins that bind HDJ1, TPR2 and MLF can be isolated, for example, by using antibody specific for HDJ1, TPR2 or MLF to immunoprecipitate HDJ1, TPR2 and

15 MLF in association with binding protein from cells. Cells expressing HDJ1, TPR2 or MLF, or that are made to express HDJ1, TPR2 or MLF, can be metabolically labeled by adding an amino acid containing a radionuclide (*e.g.*, methionine, cysteine) to the growth media. The labeled cells are lysed, immunoprecipitated with HDJ1, TPR2 or MLF antibody under conditions sufficient to allow HDJ1-, TPR2- or MLF-protein binding and fractionated, for

20 example, by SDS-PAGE, and isolated from the gel. The stringency of the immunoprecipitation conditions and/or optional wash conditions can be increased to distinguish specific from non-specific binding. Protein(s) that binds weakly to HDJ1, TPR2, or MLF can be isolated by subjecting cells to a chemical cross-linking agent prior to cell lysis or immunoprecipitation. Agents that selectively cross-link proteins in close proximity are

25 known in the art and can be chosen in order to minimize non-specific cross-linking. If desired, the binding proteins so isolated can be identified using methods disclosed herein or known in the art. Such assays can also be performed in vitro, for example, HDJ1, TPR2, or MLF affinity columns can be generated to screen for potential HDJ1, TPR2, or MLF binding proteins. The protein can then be eluted and isolated using conventional methods.

30 As used herein, the term "incubating" refers to conditions that allow contact, binding or interaction, directly or indirectly, between HDJ1, TPR2 and MLF polypeptide or

polynucleotides encoding same and the test compound. The term "contacting" includes in solution, in solid phase, in cells and in animals. As used herein, the term "binds" refers to an association, whether transient or stable, between a polypeptide and a second molecule. The term "bind" includes in solution, in solid phase in cells and in animals.

5 Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be chosen to facilitate rapid high-throughput screening.

The invention therefore provides methods for isolating a protein that binds to HDJ1, TPR2 and MLF polypeptides or functional subsequences thereof. A method includes
10 incubating at least one protein and a HDJ1, TPR2 or MLF polypeptide or subsequence thereof under conditions sufficient to allow binding; separating bound HDJ1, TPR2 or MLF polypeptide subsequence thereof from unbound HDJ1, TPR2 or MLF polypeptide or subsequence thereof and, isolating the bound protein.

A compound that modulates HDJ1, TPR2 or MLF polypeptide activity or expression
15 of a polynucleotide encoding HDJ1, TPR2 or MLF polypeptide includes "agonists," which are compounds that stimulate or activate an activity or expression and "antagonists," which are compounds that inhibit or interfere with an activity or expression. In this context,
"modulate" further includes any enzymatic interaction wherein a compound stimulates or performs a biochemical modification of a HDJ1, TPR2 and MLF polypeptide. Thus,
20 compounds that posttranslationally alter HDJ1, TPR2 and MLF, such as to increase or decrease phosphorylation, ubiquitination, glycosylation, proteolytic cleavage and the like are therefore included.

Compounds can function either directly or indirectly to modulate polypeptide activity
or expression of a polypeptide encoding polynucleotide. For example, a competitive
25 antagonist that binds HDJ1, TPR2 or MLF may directly prevent binding or participation in the signaling pathway that modulates polyglutamine toxicity. In contrast, a compound that functions indirectly may act through an intermediary molecule to achieve its agonist or antagonist affect on HDJ1, TPR2 or MLF activity or expression.

Compounds that modulate activity or expression are identified by determining
30 activity or polynucleotide expression in the presence and in the absence of a test compound. HDJ1, TPR2 and MLF biological activities or HDJ1, TPR2 and MLF expression, as

disclosed herein, can be determined using cell free systems, in cells and in a whole organism. Compounds that modulate HDJ1, TPR2, and MLF expression can be identified by detecting expression of a reporter gene operatively linked to a HDJ1, TPR2, or MLF expression control element (e.g., functional analysis of a sequence in any of SEQ ID NOs:9, 10 or 11 or a 5 human homologue). Such elements can be isolated and operatively linked to a reporter gene which provides a detection signal that reflects the amount of transcript or protein product produced. Compounds that modulate expression of a polynucleotide encoding HDJ1, TPR2 or MLF can therefore be identified by detecting expression of the reporter gene. A compound "stimulates" HDJ1, TPR2 or MLF expression if the detection signal provided by 10 the reporter gene is increased as compared with the signal in the absence of the test compound. A compound "inhibits" HDJ1, TPR2 or MLF expression if the signal is decreased as compared with the signal in the absence of the test compound. For example, cells capable of expressing HDJ1, TPR2 and MLF that have an appropriate reporter gene can be treated with a test compound, and the detection signal produced in the presence and 15 absence of the compound is determined.

Thus, the invention provides cell-based and *in vitro* methods to screen for novel binding proteins (e.g., transactivating factors) using the polynucleotides of the invention. In addition to the described cell based reporter assays, many other assays are available that screen for nucleic acid binding proteins and all can be adapted and used. A few illustrative 20 examples include, for example, mobility shift DNA-binding assays, methylation and uracil interference assays, DNase and hydroxy radical footprinting analysis (*in vitro* or *in vivo*), fluorescence polarization, and UV crosslinking or chemical cross-linkers.

One technique for isolating co-associating proteins, including nucleic acid and 25 DNA/RNA binding proteins, includes use of UV crosslinking or chemical cross-linkers, including cleavable cross-linkers dithiobis(succinimidylpropionate) and 3,3'-dithiobis (sulfosuccinimidyl- propionate); see, e.g., McLaughlin, *Am. J. Hum. Genet.* **59**:561-569 (1996); Tang, *Biochemistry* **35**:8216-8225 (1996); Lingner, *Proc. Natl. Acad. Sci. U.S.A.* **93**:10712 (1996); and Chodosh, *Mol. Cell. Biol.* **6**:4723-4733(1986).

Mobility shift DNA-protein binding assay using nondenaturing polyacrylamide gel 30 electrophoresis is an extremely rapid and sensitive method for detecting specific polypeptide binding to DNA (see, e.g., Chodosh (1986) *supra*, Carthew, *Cell* **43**:439-448(1985); Trejo, *J.*

Biol. Chem. **272**:27411-27421 (1997); and Bayliss, *Nucleic Acids Res.* **25**:3984-3990 (1997)). Interference assays and DNase and hydroxy radical footprinting can be used to identify specific residues in the nucleic acid protein-binding site, see, e.g., *Bi, J. Biol. Chem.* **272**:26562-26572(1997); Karaoglu, *Nucleic Acids Res.* **19**:5293-5300 (1991). Fluorescence polarization is a powerful technique for characterizing macromolecular associations and can provide equilibrium determinations of protein-DNA and protein-protein interactions. This technique is particularly useful (and better suited than electrophoretic methods) to study low affinity protein-protein interactions, see, e.g., Lundblad, *Mol. Endocrinol.* **10**:607-612 (1996).

10 Proteins identified in by these techniques can be further separated on the basis of their size, net surface charge, hydrophobicity and affinity for ligands. In addition, antibodies raised against such proteins can be conjugated to column matrices and the proteins immunopurified. All of these general methods are well known in the art (see e.g., Scopes, R. K., Protein Purification: Principles and Practice, 2nd ed., Springer Verlag, (1987)).
15 Chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

20 As described herein, MLF expression is likely to be linked to particular types of human cancers (e.g., myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)). Thus, compounds can be screened for their effect on activity or expression of MLF and such compounds are likely to be therapeutically useful in treating patients suffering from myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML).

25 Transgenic flies that carry dMLF cDNA, dMLF cDNA as P-element chromosomal insert, UAS-containing P-elements inserted upstream of dmlf gene, or protein products of dMLF cDNA, are also useful for this purpose. Nuclear localization of a large portion of MLFin NPH-MLF fusion product appears required for its pro-apoptotic effect, and perhaps for its effect on cell proliferation. Therefore, to produce a similar phenotype, dMLF may be fused to a nuclear localization signal (dMLF-NLS) to allow the delivery of dMLF into the nucleus. A *Drosophila* model containing such an MLF chimera can exhibit a measurable phenotype such as early death or external tumor growth. Alternatively, dMLF can be fused 30 to the fly homologue of nucleophosmin to generate a fusion protein similar to NPH-MLF and expressed in the animal. In any case, such dMLF chimeras can be expressed in various

tissues and cells of the animal to determine its effect in different tissues and cells and to produce a suitable animal model for identifying genes and compounds that modulate MLF activity or expression.

Alternatively, over-expressing dMLF may produce a phenotype and such animals can be employed in the screen. As it is likely that dMLF is involved in a molecular cascade with several protein components, over-expression of dMLF will disrupt the normal stoichiometry between the various components of the pathway and produce a phenotype that can be used to identify modulatory genes or compounds as described herein. Subsequently, genetic partners of dMLF pathway are potential targets for therapeutic agents in treating patients with MDS, AML, and other forms of cancer related to MLF pathway.

Thus, compounds that regulate MLF activity or expression are likely to be useful as therapeutics for treating these and other cancers associated with MLF. Thus, in accordance with the invention, there are provided methods of identifying compounds that modulate MLF activity or expression as described herein for polyglutamine toxicity. Such an approach also is applicable to TPR2.

Chimeras comprising HDJ1, TPR2 or MLF, or subsequences, and a heterologous sequence from another protein (*e.g.*, GAL4, VP16 DNA binding (DBD), activation domains (AD), and the like) also can be used to identify compounds that modulate a HDJ1, TPR2, or MLF activity in cells. Chimeras having particular HDJ1, TPR2 or MLF subsequences are useful for identifying genes or compounds that modulate activities conferred by the subsequence.

For example, to identify genes or compounds that modulate a HDJ1, TPR2 or MLF activity, a chimera comprising HDJ1, TPR2 or MLF, and a GAL4 DNA binding domain (GAL4_{DBD}) can be expressed in cells. A library of polynucleotides linked to an activation domain also present in the cells allows a protein encoded by a polynucleotide of the library to interact with HDJ1, TPR2 or MLF. A sufficiently strong interaction between HDJ1, TPR2 or MLF and an interacting protein will activate transcription of the reporter gene driven by the GAL4 response element. Once identified, the assay can be extended further to identify compounds that modulate the interaction by adding a test compound and assaying for levels of reporter expression in the presence and absence of the test compound. Yeast and mammalian two-hybrid cell systems are well known in the art, are commercially available,

and are therefore applicable in the methods for isolating and/or identifying binding proteins and those that modulate activity.

The signal provided by the reporter gene can be, for example, RNA, protein, an enzymatic activity, and the like. Thus, the signal can be detected by a variety of methods known in the art, including northern analysis, RNA dot blots, nuclear run-off assays, ELISA or RIA, Western blots, SDS-PAGE alone, or in combination with antibodies that immunoprecipitate the reporter gene product. Expressed products that provide an enzymatic activity or detection signal are preferred and include, for example, β -galactosidase, alkaline phosphatase, horseradish peroxidase, luciferase, green fluorescent protein, and chloramphenicol acetyl transferase. Cells contemplated for use in these methods include the cells described herein, for example, insect cells, mammalian cells (e.g., CV-1, COS, HeLa and L-cells), yeast cells and bacteria.

The invention further includes heterologous functional domains that facilitate entry of a modulatory gene (e.g., HDJ1, TPR2, MLF) into a cell. One example of such a heterologous functional domain that facilitates entry into a cell is a ligand to a cell surface receptor. Additional heterologous domains that provide a cell targeting function or facilitate cellular entry also are known to those skilled in the art. Such domains include, for example, viral capsid proteins, retroviral envelope proteins, a natural or engineered viral protein with a desired cell tropism.

A heterologous functional domain also can decrease or increase the activity of the genes identified by the methods of the invention. To increase activity of a gene that increases polyglutamine toxicity, domains which exhibit apoptotic, cell cycle arrest or delay, cytotoxic or cytostatic activity can be included, for example, ligands or agonists to receptors that induce apoptosis. Fas ligands or anti-Fas antibodies are two specific examples of such apoptotic domains. Domains that exhibit cytotoxic or cytostatic activity include, for example, toxins and chemotherapeutic agents such as doxorubicin, methotrexate, vincristine, and cyclophosphamide can be conjugated to a polypeptide. Other agents exist and are known to those skilled in the art and can be linked to enhancer genes to augment their cell toxicity. For example, genes required for cell proliferation or cell cycle progression can be inhibited by a heterologous antisense nucleic acid of that gene. Cell cycle arrest can be stimulated by a

negative regulator of cell growth, for example, a growth suppressor gene such as Rb, p53, DPC, etc.

Heterologous functional domains also include regulatable moieties that modulate activity of a polypeptide identified by a method of the invention. When linked to a HDJ1, 5 TPR2, MLF polypeptide, a modular domain can impart ligand-dependent activation or repression of its polyglutamine toxicity decreasing activity. Various different ligand-dependent transcription factors having inducible ligand-binding domains are known in the art are applicable in such chimeras.

A heterologous functional domain also can provide a variety of other useful functions 10 known to those skilled in the art. For example, it can be a lipid-based agent to facilitate cell entry, or an agent that increases or decreases the stability of the HDJ1, TPR2, MLF polypeptide and subsequences thereof either intra- or extra-cellularly.

A heterologous functional domain also can provide an imaging and/or visualization 15 function that is mediated by an isotopic, calorimetric, or fluorometric agent. Such an imaging function is useful for screening an expression library for interacting proteins, or for detecting or localizing apoptosis *in vivo*. As exemplified herein, a hemagglutinin tag is but one example of a tag (epitope tag) that can be used to detect or visualize the presence of the tagged protein in animal tissue sections. Additional examples include myc, Flag, GFP, T7, polyhistidine and DNA polymerase.

20 Polypeptides and polynucleotides also can contain multiple heterologous functional domains. For example, a gene that increases or decreases polyglutamine toxicity can be operatively linked to two or more identical or two or more different domains or moieties. An example of such a configuration would be a molecule containing two or more different domains, a cell targeting domain and a chemotherapeutic moiety, operatively linked to a gene 25 that increases polyglutamine toxicity. The exact chemical nature and structural organization of such a molecule will be known to those skilled in the art and can be determined based on the particular application.

A heterologous functional domain can consist of a variety of different types of 30 moieties ranging from small molecules to large macromolecules. Such moieties can be, for example, nucleic acid, polypeptide or peptide, carbohydrate, lipid, or small molecule

compounds. Both natural and non-naturally occurring compounds and derivatives are similarly included.

Test compounds for use in the screening methods of the invention are found among biomolecules including, but not limited to: peptides, polypeptides, peptidomimetics, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Test compounds further include chemical compounds (e.g., small organic molecules having a molecular weight of more than 50 and less than 5,000 Daltons, such as hormones). Candidate organic compounds comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate organic compounds often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Known pharmacological compounds are candidates that may further be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidation, etc., to produce structural analogs.

Test compounds can additionally be contained in libraries, for example, synthetic or natural compounds in a combinatorial library; a library of insect hormones is but one particular example. Numerous libraries are commercially available or can be readily produced; means for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides, also are known. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or can be readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Such libraries are useful for the screening of a large number of different compounds.

A variety of other compounds may be included in the screening method. These include agents like salts, neutral proteins, e.g., albumin, detergents, etc. that are used to facilitate optimal protein-protein binding or interactions and/or reduce nonspecific or background binding or interactions *in vitro*. For example, reagents that improve the

efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., may be used.

Genetic elements and other compounds that decrease polyglutamine toxicity are useful in treating polyglutamine associated and polyglutamine related disorders characterized by cell degeneration, death, apoptosis, protein aggregation (in nucleus, cytoplasm in extracellular), misfolding, deficient or aberrant protein transport or degradation, etc., as set forth herein. Genetic elements and other compounds that increase polyglutamine toxicity are useful in treating cell proliferative disorders, or disorders associated with undesirable cell survival, cell growth or cell differentiation. For example, almost all cells express polypeptides that contain polyglutamine repeat sequences. Thus, by increasing cell sensitivity to polyglutamine repeat sequence toxicity, such polyglutamine repeat containing polypeptides may be rendered toxic. In this way, such cells would be rendered susceptible to polyglutamine toxicity by introducing a gene or contacting with a compound that increases polyglutamine toxicity. For example, a compound having an ability to decrease cell survival can be cell death or apoptosis inducer and can be useful in the therapeutic methods of the invention for treating cell proliferative disorders or disorders characterized by undesirable cell growth or survival.

Accordingly, as the invention provides animal models and screening methods useful for identifying classes of genes and compounds that increase and decrease toxicity the identified genes and compounds that increase or decrease cell survival, growth, proliferation, differentiation, apoptosis, development or viability, behavioral abnormalities, neuron excitability, protein aggregation, misfolding, transport, or degradation, the methods and identified genes and compounds have obvious therapeutic applications for identifying and treating disorders treatable by increasing or decreasing one or more of the aforementioned cellular or tissue effects.

Thus, the invention also provides genes and compounds that increase or decrease cell survival, growth, proliferation, differentiation, apoptosis, protein aggregation, protein misfolding, protein transport, protein degradation in a pharmaceutically acceptable carrier. In one embodiment, a composition of the invention includes a TPR2 polynucleotide and a pharmaceutically acceptable carrier. In another embodiment, a composition of the invention includes a TPR2 polypeptide and a pharmaceutically acceptable carrier. In yet another

embodiment, a composition of the invention includes a MLF polynucleotide and a pharmaceutically acceptable carrier. In still another embodiment, a composition of the invention includes an MLF polypeptide and a pharmaceutically acceptable carrier. In particular aspects, TPR2 and MLF are mammalian, such as human, bovine, porcine, equine or ungulate sequence, or an insect (*e.g.*, *Drosophila*) sequence. In additional aspects, the polynucleotide is operatively linked to an expression control element.

Polyglutamine related or polyglutamine like disorders are generally caused by pathological conditions characterized by protein aggregates (intracellular, in nucleus or cytoplasm, or extracellular), abnormal or enhanced cell degeneration, death or apoptosis, decrease cell survival, proliferation or differentiation, and the like can be treated by the genes and compounds of the invention and identified by the methods of the invention. Thus, the invention further provides methods of modulating polyglutamine toxicity or a polyglutamine like disorder in a cell. In one embodiment, a method of the invention includes contacting a cell with a gene or compound that modulates polyglutamine toxicity. In one aspect, the cell is *in vitro*. In another aspect, the cell is *in vivo*. In additional aspects, the cell is a neuron, retina, muscle or mesoderm cell.

In another embodiment, the cell is contacted with a J domain-containing gene. In one aspect, the gene is selected from HDJ1 or TPR2. In another aspect, the cell is a neural, retinal, muscle or mesoderm cell. In other aspects, the cell is contacted with a J domain gene, HDJ1, TPR2, or MLF gene antisense polynucleotide.

Polyglutamine disorders typically share features in common with other degenerative, cell death or apoptotic, decreased cell survival, growth or proliferation, and protein aggregative, folding, transport and degradative disorders. Such disorders are referred to herein as polyglutamine “related disorders,” or polyglutamine “like disorders.” The features frequently found to be in common among these disorders include cellular degeneration or atrophy, protein aggregation with or without protein accumulation in nucleus and/or cytoplasm of the cell, deficient or decreased protein folding or transport, increased cell death or apoptosis, decreased cell viability, growth or differentiation, and formation of intracellular or extracellular plaques. Accordingly, due to the common features that characterize such disorders, it is anticipated that the genes and other compounds that modulate polyglutamine toxicity identified will also modulate cellular degeneration or atrophy, protein aggregation,

aggregate accumulation in nucleus and/or cytoplasm of the cell, development or viability, behavioral abnormalities, neuron excitability, deficient or decreased protein folding or transport, increased cell death or apoptosis, decreased cell viability, growth or differentiation, or formation of intracellular or extracellular plaques, whether or not the particular conditions 5 are due to expression of an expanded polyglutamine repeat sequence. Thus, genes or compounds that directly or indirectly modulate cellular degeneration or atrophy, development or viability, behavioral abnormalities, neuron excitability, protein aggregation, aggregate accumulation in nucleus and/or cytoplasm of the cell, protein folding or transport, cell death or apoptosis, cell viability, growth or differentiation, or formation of intracellular or 10 extracellular plaques, whether or not the particular conditions are due to expression of an expanded polyglutamine repeat sequence, can therefore be identified using the methods of the invention. Accordingly, diseases characterized by apoptosis independent of polyglutamine sequence can be treated by using any of the described methods for treating polyglutamine toxicity.

15 Thus, the invention further provides methods of increasing cell survival. A method includes contacting a cell with an amount of a gene or compound that increases cell survival. In one embodiment, the cell is *in vitro*. In another embodiment, the cell is *in vivo*. In yet another embodiment, the cell is contacted with a gene or a polypeptide encoding the gene or compound that decreases polyglutamine toxicity. In still another embodiment, the cell 20 exhibits polyglutamine toxicity.

In still another embodiment, the cell has or is at risk of degeneration, atrophy, protein aggregation with or without accumulation in nucleus and/or cytoplasm of the cell, deficient or decreased protein folding or transport, cell death or apoptosis, decreased cell viability, growth or differentiation, and developing intracellular or extracellular plaques. In one aspect, the 25 gene comprises a J domain-containing gene. In another aspect, the gene is selected from HDJ1, TPR2, and MLF. In yet another aspect, the cell is a neural, retinal, muscle or mesoderm cell.

The invention additionally provides methods of decreasing cell death or apoptosis. A method includes contacting a cell with an amount of a gene or compound that decreases cell 30 death or apoptosis. In one embodiment, the cell is *in vitro*. In another embodiment, the cell is *in vivo*. In yet another embodiment, the cell is contacted with a gene or a polypeptide

encoding the gene or compound that decreases polyglutamine toxicity. In still another embodiment, the cell has or is at risk of degeneration, atrophy, protein aggregation with or without accumulation in nucleus and/or cytoplasm of the cell, deficient or decreased protein folding or transport, cell death or apoptosis, decreased cell viability, growth or 5 differentiation, and developing intracellular or extracellular plaques. In still another embodiment, the cell exhibits polyglutamine toxicity. In one aspect, the gene comprises a J domain-containing gene. In another aspect, the gene is selected from HDJ1, TPR2 and MLF. In yet another aspect, the cell is a neural, retinal, muscle or mesoderm cell.

Methods of decreasing polyglutamine toxicity in a tissue or organ of a subject having 10 or at risk polyglutamine toxicity also are provided. A method of the invention includes contacting the tissue or organ with an amount of a J domain containing polypeptide, a TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, to decrease polyglutamine toxicity in the tissue or organ of the subject. In one embodiment, the tissue is brain, eye, muscle or 15 mesoderm.

Methods of decreasing the severity of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in a subject having or at risk of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder also are provided. A method of the invention includes administering to the subject 20 an amount of J domain containing polypeptide, a TPR2, or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, to decease the severity of the frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in the subject. In one embodiment, the disorder is a neurological or muscle disorder. In another embodiment, the disorder 25 impairs long term or short-term memory or coordination of the subject. In still another embodiment, the disorder is associated with polyglutamine toxicity.

In yet another embodiment, the disorder is characterized by the presence of protein aggregates, amyloid plaques, degeneration or atrophy in an affected tissue or organ. In still other embodiments, the disorder is selected from the group consisting of Alzheimer's 30 disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD),

Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma. The severity of such disorders can be decreased by decreasing cell death or by decreasing protein aggregation, for example.

In additional embodiments, the methods of the invention include treating the various 5 disorders or conditions herein by prophylactic administration.

Apoptosis participates in the maintenance of tissue homeostasis in a number of physiological processes such as embryonic development, hematopoietic cell regulation, and normal cell turnover. Dysfunction, or loss of regulated apoptosis, can lead to a variety of pathological disease states. For example, the loss of apoptosis can lead to the pathological 10 accumulation of self-reactive lymphocytes, hyperproliferative cells, such as neoplastic or tumor cells, virally infected cells and cells that contribute to fibrotic conditions.

Inappropriate activation of apoptosis also can contribute to a variety of pathological disease states, including, for example, acquired immunodeficiency syndrome (AIDS), neurodegenerative and musculardegenerative diseases, and ischemic injury. Treatments 15 designed to modulate the apoptotic pathways in these and other pathological conditions can alter the progression of many of these diseases.

The invention therefore also provides methods of identifying genes or compounds that modulate apoptosis or cell death. Such genes and compounds include those useful for treating neoplastic, malignant, autoimmune, or fibrotic pathological conditions. A method 20 of the invention included is essentially as set forth for the methods for identifying modulators of polyglutamine toxicity.

As the invention chimeric polypeptides, polynucleotides and antibodies will be administered to subjects, including humans, the present invention also provides pharmaceutical formulations comprising invention polypeptides, polynucleotides and 25 antibodies. The compositions administered to a subject will likely be in a "pharmaceutically acceptable" or "physiologically acceptable" formulation. As used herein, the terms "pharmaceutically acceptable" and "physiologically acceptable" refer to biologically compatible carriers, diluents, excipients and the like that can be administered to a subject, preferably without excessive adverse side effects (e.g., nausea, headaches, etc.). Such 30 preparations for administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol,

polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present, such as antimicrobial, anti-oxidants, chelating agents, and inert gases, for example. Various pharmaceutical formulations appropriate for administration to a subject are known in the art are applicable in the methods of the invention (*e.g.*, Remington's Pharmaceutical Sciences, 10 18th ed., Mack Publishing Co., Easton, PA (1990); The Merck Index, 12th ed., Merck Publishing Group, Whitehouse, NJ (1996)).

Pharmaceutically acceptable formulations further include compositions where the duration of action or delivery of an administered composition is controlled. Such formulations include particles or a polymeric substance such as polyesters, polyamine acids, 15 hydrogel, polyvinyl pyrrolidone, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers. The rate of release of the composition may be controlled by altering the concentration or composition of the macromolecules. For example, it is possible to entrap a polynucleotide or polypeptide in 20 micro-capsules prepared by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly (methylmethacrolate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, 25 microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

The compositions administered by a method of the invention can be administered parenterally by injection, by gradual perfusion over time, or by bolus administration (for example, or by microfabricated implantable device. The composition can be administered intracranially, intravenously, intramuscularly, intraperitoneally, subcutaneously, intracavity, 30 via inhalation, transdermally, or intravascularly. The compositions can be administered in multiple doses or at multiple sites in the same or in different amounts. The composition can

be administered to a subject at the site of the pathology (e.g., the brain, muscle, etc.). For the treatment of a neoplastic or undesirable cell growth or proliferative disorder, the composition can be administered by direct injection into a solid tumor mass or into a region of fibrosis. The active ingredient can enter the tissue by passive diffusion or, alternatively, by a delivery vehicle (e.g., a lipid-based vesicle is one example of a delivery vehicle).

The “effective amount” will be sufficient to decrease, prevent, or ameliorate polyglutamine toxicity, a polyglutamine related disorder, or any of the biological or pathophysiological features that characterize such disorders as described herein or known in the art. The doses sufficient to provide an “effective amount” for treating, decreasing or improving polyglutamine toxicity will be sufficient to ameliorate or improve one or all of the symptoms of the condition, although preventing a progression or worsening of the condition is a satisfactory outcome for many conditions. The concentration of the aforementioned compositions required to be effective will depend on the organism targeted and the formulation of the composition and the ameliorative effect desired (*i.e.*, increased or decreased toxicity). For example, an effective amount of a composition is that amount sufficient to cause a reduction in polyglutamine toxicity, as determined using any of the parameters described herein (e.g., decreased cell degeneration, death or apoptosis, increased cell survival, proliferation, differentiation, viability or development, decreased behavioral abnormality, decreased protein aggregation, increased protein transport or folding, etc.). As the various cellular, biological, morphological, phenotypical and behavioral effects of polyglutamine toxicity are disclosed herein, or otherwise known in the art, the effect of a gene or compound on each of these elements individually, or in any combination, can be conveniently determined in order to ascertain an effective amount. Introduction of the invention compositions into a sufficient number of diseased cells of the subject can inhibit or decrease toxicity or improve any of these parameters thereby altering the course of the pathology.

Thus, for treating Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD), Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma, for example, treatment can be initiated at an early or mid-level progressive stage. An inhibition, delay or decreased

worsening of the condition is a satisfactory clinical outcome. Doses sufficient to treat cell proliferative disorders, or conditions characterized by abnormal or undesirable cell survival, proliferation or differentiation will be sufficient to delay proliferation or differentiation, for example, by arresting or delaying progression through the cell cycle. Again, an inhibition or 5 delay of cell growth or proliferation, or preventing a worsening of the condition (for example, by slowing growth of a tumor, by slowing metastasis of the tumor) is considered a satisfactory clinical outcome. An effective amount can readily be determined by those skilled in the art (see for example, Ansel *et al.*, "Pharmaceutical Drug Delivery Systems," 5th ed. (Lea and Febiger (1990), Gennaro ed.)).

10 In accordance with the present invention, there are provided kits containing the compositions of the invention. In one embodiment, a kit of the invention contains one or more J domain containing, HDJ1, TPR2, or MLF polypeptides, functional subsequences thereof, antibodies that specifically bind to the polypeptides, or J domain, HDJ1, TPR2, and MLF encoding polynucleotides, and a label or packaging insert in suitable packaging material. In one embodiment, the label or insert includes instructions for treating a disorder 15 as described herein by administering J domain containing, HDJ1, TPR2, or MLF polypeptides, or J domain, HDJ1, TPR2, and MLF encoding polynucleotides. In one aspect, the kit contains a human TPR2 or MLF encoding polynucleotide operatively linked to an expression control element in a pharmaceutically acceptable carrier, and a label or insert with 20 instructions for treating polyglutamine toxicity or a polyglutamine related disorder, as described herein.

In another embodiment, the label or insert includes instructions for detecting TPR2 or MLF in a biological sample (*e.g.*, neural tissue, eye tissue, muscle or mesoderm) having or suspected of having or developing polyglutamine toxicity or a polyglutamine related disorder, as described herein. 25

In yet another embodiment, the kit contains a transgenic animal of the invention. In one aspect, the transgenic animal comprises a *Drosophila* that includes a polyglutamine repeat sequence encoded by a plurality of CAGs and at least one CAA that exhibits polyglutamine toxicity, and a label or insert including instructions for maintaining the animal. In one aspect, the kit additionally contains instructions for identifying modulators of 30 polyglutamine toxicity.

As used herein, the term "packaging material" refers to a physical structure housing the components of the kit, such as invention polypeptides, antibodies, polynucleotides and animals. The packaging material can maintain the components steriley, and can be made of material commonly used for such purposes (e.g., paper, corrugated fiber, glass, plastic, foil, etc.). The label or packaging insert can indicate that the kit is to be used in a method of the invention, for example.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

All applications, publications, patents, other references, GenBank citations and ATCC citations mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims. The invention is further described in the following examples, which do not limit the scope of the invention(s) described in the claims.

EXAMPLE 1

This example describes various materials and methods used in the studies.

Production of Transgenic Flies

Flies were maintained on cornmeal/yeast/agar at 25°C and 70% humidity. Transgenic constructs were prepared for microinjection as follows: 13.5 μ g transgenic vector, 4.5 μ g p π transposase vector, 0.1M sodium phosphate buffer (pH 7.8), 5mM potassium chloride, in 25 50 μ l aqueous solution. Using Transjector 5246 and Femtotips II (Eppendorf), the transgenic constructs were microinjected into 5-30 min. old, fertilized w^{1118} fly eggs. Several transgenic lines for each were established. Since the expression of the UAS transgenes requires activation by a GAL4-expressing driver, these lines had no obvious phenotypes and were easily maintained.

Sections and Antibody Fluorescent Labeling

Fly heads were placed in OCT 4583 embedding medium (Sakura Finetek) and horizontal sections were prepared with Tissue-Tek II using Leica knives and transferred onto Superfrost/Plus microscope slides (Fisher Scientific). Slides were dried on a 50°C hot plate for 30 sec and sections were fixed in Mirsky's fixative (National Diagnostics) for 30 min. at room temperature. After washing 3 times within 10 min. using PBS/Tween20 (0.1%), sections were blocked in a PBS/bovine serum albumin fraction V (1%) (Sigma) solution and incubated with 1 µg/ml of primary polyclonal antibody (Y-11, Santa Cruz Biotechnology, Inc.) in the solution for 2 hrs. at room temperature. The sections were washed 3 times, 5-min each, with PBS/Tween20 (0.1%), then incubated with 4 µg/ml of FITC-labeled secondary anti-rabbit antibody (Jackson ImmunoResearch Laboratories) in the solution for 1 hr at room temperature. The sections were washed for 5-min with PBS/Tween20 (0.1%), covered with DAPI for 1 min., and washed 3 times (15-min. each) with PBS/Tween20 (0.1%). Finally, the sections were mounted in 0.1 mg/ml phenyl diamine (PDA)/0.5 µg/ml 4'-6' diamino-2-phenylindole (DAPI)/90% glycerol mounting solution. The immunofluorescence-labeled sections were photographed on a Zeiss axioplan microscope with an MC100 camera, using Kodak 100 ASA color films.

Scanning Electron and Light Microscopy

Adult flies, 1-6 hours old, were anaesthetized by ether for 1-2 min. and attached by their backs to stubs with adhesive, placed in the vacuum chamber of ETEC scanning electron microscope, and photographed within 10 min. For light microscopy, adult flies, 1-6 hours old, were etherized for 2-3 min., placed on their side on the white strip of RITE-ON micro slides (Gold Seal Products) and photographed by Leica MZFLIII dissecting microscope, illuminated by two sets of optic fiber illuminators (Ehrenreich Photo Optical Industries or Cole Parmer Instrument Company), using Fuji 1600 ASA Super HG color film. Prints were scanned on Lacie Silverscanner III with Adobe Photoshop 5.0 at 300 dpi and processed on graphics software Canvas 5.0.3.

30 Identification of Genes Modulating Polyglutamine by Plasmid Rescue

Plasmid rescue (Pirrotta (1986); Pirrotta, Cloning Drosophila Genes: A Practical Approach, pp 83-110, IRL Press, Oxford, Washington, D.C., ed. D.B. Roberts (1986)) was done with the following modification: from an established line, genomic DNA was isolated by QIAamp Tissue kit (Qiagen) and digested with 6 restriction enzymes: BfI, BglII, EcoRI, HincII, SacI, and SacII in 100 μ l reaction volume overnight. Digested fragments were purified by QIAprep Spin Miniprep kit (Qiagen), circularized by ligation in 50 μ l reaction and transformed by electroporation of 1.5 μ l of ligation reaction into the DH10B (Gibco/BRL) strain of *E. coli*. Colonies carrying the P-element were selected by plating transformed bacteria on media with Kanamycin. DNA was isolated from positive colonies and the approximate size of the insert (flanking genomic DNA) determined by Aval restriction enzyme digestion. Inserts of sufficient size were sequenced by automated sequencing and the results were compared with known DNA or protein sequences in the database by Berkeley Drosophila Genome Project (BDGP) BLAST server (BLASTN) and The Baylor College of Medicine Search Launcher (BLASTP+BEAUTY). Protein alignments were performed by MacVector PPC 6.0.1 application software. Program parameters for *Drosophila* dTPR2 and human TPR2 were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 10.0: Extend gap penalty 0.1; similarity matrix blosum. For *Drosophila* dMLF and human MLF the program parameters were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 1.0: Extend gap penalty 0.1; similarity matrix blosum. EST search parameters were BLASTN 2.0a19MP

Cloning of Suppressor Genes

The cDNA containing the coding region of dHDJ1 was removed from GH26396 (contained in the plasmid pOT2a, obtained from Research Genetics, Inc.) by complete digestion of 2.5 μ g of plasmid DNA, in NEB #2 restriction enzyme buffer and 0.1 mg/ml BSA (New England Biolabs), with 20 u HindIII for 3 hrs at 37°C to fragment pOT2a backbone, followed by partial digestion with 1, 2 or 4 u of PstI and XhoI for 10 min. at 37°C, and enzyme inactivation at 65°C for 10 min. The reactions were run on 1% agarose gel and a 1816-bp-fragment was isolated and purified by QIAquick gel extraction kit (Qiagen). This fragment, which contains 106 bp PstI/EcoRI fragment of pOT2a, 11 bp upstream of the

reported 5'UTR, the 5'UTR, dHDJ1 ORF, 406 bp of the 579 bp reported 3'UTR, and a 23-bp-long poly(A), was ligated into the transgenic vector pINDY6 PstI/XhoI site.

For cloning dTPR2, the PstI/XhoI fragment containing 106 bp PstI/EcoRI fragment of pOT2a, the 365-bp 5'UTR, the 1527-bp dTPR2 ORF, the 328-bp 3'-UTR, and a 20-bp-long poly(A) was removed from GH09432 (within pOT2a) and ligated into the transgenic vector pINDY6 PstI/XhoI site.
5

For cloning dMLF cDNA, the PstI/XhoI fragment of GH20101 in pOT2a plasmid (Research Genetics, Inc.) containing dMLF orf and its 5' and 3' UTR was removed and ligated into the transgenic vector pINDY6 PstI/XhoI site.

10

EXAMPLE 2

This example describes the construction of polyglutamine repeat sequence expression vectors and *Drosophila melanogaster* that express variously sized polyglutamine repeat sequences. This example also describes the generation of P-element insertion flies used for screening for genetic elements that modulate polyglutamine toxicity.
15

Polyglutamine sequences encoded by short (20), intermediate (63) and expanded (127) CAG tracts interspersed with CAA were synthesized using a modified version of a polymerase chain reaction (PCR) method (Kazemi-Esfarjani *et al.*, *Hum. Mol. Genet.*, 4:523-527 (1995)). Briefly, the fly *prospero* gene, in the p139cAC1 plasmid (Robertson *et al.*,
20 *Genetics*, 118:461-470 (1988)), was used as a template because it has a polyglutamine encoding tract of 20 repeats. The primers used for PCR to amplify two fragments were:
ProsBamHI3229F (5'-ATG CGC GGA TCC CAG CAG CTG GAG CAG AAC GAG GCC-3') with ProsAflIIR (5' phosphorylated) (5'-ATT GCT GTT GCC GCC GTT CTT AAG CTG TTG TTG TTG CTG TTG TTG-3') and ProsBstBIF (5'-ACC GGA GGC CCA CCG
25 TCA TTC GAA CAG CAG CAG CAA CAG-3') with Pros3650R (5'-GCT GCG TGC GGA TTG AAG AAC GGC-3'). The reaction mixture contained 100ng p139cAC1 template, 50pmol of each primer, 1X cloned Pfu buffer (Stratagene), 0.2 mM dNTP, 5% glycerol, 5% dimethyl sulfoxide (DMSO), and 1.25 unit cloned Pfu DNA polymerase (Stratagene) in a total volume of 62 µl aqueous solution, overlaid with mineral oil. PCR was performed with a
30 Stratagene Robocycler Gradient 96 in 200-µl thin-walled tube strips. The thermal cycling parameters were denaturation at 95°C for 3 min., for one cycle, followed by 35 cycles of

denaturation at 95°C for 30 sec., annealing at 65°C-80°C for 1 min., extension at 75°C for 1 min., and finally, extension at 75°C for 10 min.

The PCR products were digested with BamHI (5'fragment) or BstXI (3' fragment) and ligated (T4 DNA ligase, Gibco/BRL) into p139cAC1 digested with BamHI/BstXI. After 5 cloning and amplifying this construct in XL1 Blue strain of *E. coli* (Stratagene), the sequence between the two polyCAG tracts was removed by digestion with BstBI and AflII (or BfrI), blunt-ended with Mung bean nuclease (New England Biolabs) followed by ligation and transformation into XL1 Blue. To synthesize polyglutamine of 63 repeats, this procedure was repeated twice, an additional time (3X) for 127 repeats, an additional time for 190 (4X) 10 and an additional time (5X) for 223 repeats.

To produce the hemagglutinin (HA) tagged polyglutamine sequence driven by a yeast upstream activating sequence (UAS), UAS-20QHA, UAS-63QHA and UAS-127QHA, the polyglutamine encoding and flanking nucleotide sequences were PCR-amplified as above with primers 5'Gln2F (5'-CGG AAT TCG CCG CCA CCA TGG GAG GCC CAC CGT 15 CAA CCC CCC AGC AG-3') and 3'GlnR (5'-ATT GCT GTT GCC GCC GTT ACT AGT CTG TTG CTG CTG CTG TTG-3'). The PCR product was digested with EcoRI and SpeI and, by using a PstI-EcoRI adaptor, inserted in-frame with a hemagglutinin tag DNA sequence into PstI/SpeI digested pINDY6 transgenic vector (a pUC 19 backbone containing a miniwhite gene, an ampicillin-resistance gene, and 5 tandem upstream activating sequences 20 (UAS), followed by a minimal hsp70 promoter, a polyclonal site, a SV40 polyA signal, and 5' and 3' P-elements). The resulting plasmids express the polyglutamine repeat flanked by 8 amino acids on the N-terminal side and 13 amino acids on the C-terminal side (MGGPPSTPQ_nTSRTYPYDVPDYA; Figure 1B). Figure 2 shows a schematic of P-element constructs having variously sized HA-tagged polyglutamine repeat sequences.

25 Several transgenic lines for each polyglutamine repeat sequence were established following microinjection of fertilized *w¹¹¹⁸* fly eggs with the transgenic vector. Since expression of the UAS polyglutamine sequence transgenes requires activation by a GAL4-expressing driver, these fly lines had no obvious phenotypes.

To activate expression of the polyglutamine repeat sequences in transgenic flies, 30 genetic crosses between the transgenic polyglutamine flies and flies expressing yeast GAL4 transcription factor were produced. Yeast GAL4 was regulated by an eye-specific promoter

GMR (GLASS multiple repeats) (Spradling *et al.*, *Science*, **218**:341-347 (1982); and Pirrotta, Cloning Drosophila Genes: A Practical Approach, pp 83-110, IRL Press, Oxford, Washington, D.C., ed. D.B. Roberts (1986)) cloned upstream of the GAL4 cDNA. GMR is active in all retinal cells, from the time of their differentiation throughout adulthood. In a 5 separate set of studies, a neuron-specific driver *Appl*-GAL4 was used to express polyglutamine repeat sequences in the fly nervous system (Torroja *et al.*, *Current Biology*, **9**:489-492 (1999)).

EXAMPLE 3

10 This example describes histological and pathophysiological characteristics of polyglutamine toxicity in *Drosophila melanogaster*. This example also describes screening for genetic elements that modulate polyglutamine toxicity, and the isolation of flies that contain genetic elements which suppress and enhance polyglutamine toxicity.

Flies with a heterozygous insertion of GMR-GAL4 alone had fully developed eyes 15 (Figure 4A and 5A). When combined with chromosome carrying UAS and a short length of polyglutamine (20Q), eye development was normal for external structure and pigmentation. Using an anti-HA antibody, immunohistological examination of head cryosections of one-day-old flies carrying GAL4 alone, or GAL4 plus 20Q, revealed no polyglutamine aggregates. In contrast, flies expressing the 127 polyglutamine repeat sequence had severely 20 collapsed eyes lacking pigmentation, and, in sections, anti-HA antibody revealed abundant polyglutamine aggregates in the eye (Figure 4B and 5B). The 127 polyglutamine repeat sequence expressing flies were subsequently used to screen for genetic factors that modulate polyglutamine toxicity.

To screen for genes that modulate toxicity of 127Q, flies having random P-element 25 transpositions were *de novo* generated using the fly stock carrying the P[ry⁺, Δ2-3](99B) transposase (Robertson *et al.*, *Genetics* **118**:461-70 (1988)) and an X-linked EP insert (EP55; Rorth, *Proc. Natl. Acad. Sci. USA* **93**:12418-22 (1996)) (Figure 3). EP P-elements contained fourteen UAS sequences in tandem to enhance expression of nearby gene(s), followed by the *hsp70* heat shock minimal promoter (pEP plasmid) (Rorth, *Proc. Natl. Acad. Sci. USA* **93**:12418-22 (1996)). The mutant fly lines were generated by mobilizing the X-linked P- 30 element in the EP55 strain, and isolating those with new insertions on chromosomes 2 and 3.

In detail, homozygous EP55 virgin females were crossed with males homozygous for a defective transposon, expressing the transposase. The F1 male progeny were crossed with virgin *w1118* females (w/w). The F2 Male progeny that had coloured eyes and lacked the transposon's genetic markers were selected, as they contain a new stable P-element insertion 5 on an autosomal chromosome.

The P-elements containing 14 tandem UAS elements, which, in the presence of GAL4, drive the expression of downstream genomic sequences (Rorth, *Proc Natl Acad Sci USA*, **93**:12418-12422 (1996)). Hence, if there is a locus downstream of a P-element insertion that codes for a modifier gene, it will be activated and cause a change in the eye 10 phenotype. Once a modulator was found, a single male was crossed to female (CyO;TM3)/Xa. The resulting male progeny were crossed to *w1118* flies to separate the P-elements. This resulted in colored-eye progeny that carry a balancer for one chromosome and a P-element on another. Males from such progeny were tested for suppression or 15 enhancement of activity by crossing to female w;GMR/CyO;127Q/127Q. The lines were established by crossing the latter males to (CyO;TM3)/Xa, and by crossing the resulting flies carrying CyO and TM3 balancers. The lines that produced the expected effects were selected for further amplification and plasmid rescue.

Seven thousand randomly generated P-element insertion strains were crossed with GMR-GAL4/UAS-127Q flies, and the F1 progeny were assessed for either suppression or 20 enhancement of the eye phenotype. Among the 7000 P-element insertion strains screened, 30 suppressor and 29 enhancer lines were identified that either suppressed or enhanced the polyglutamine-dependent eye degeneration of GMR-GAL4/UAS-127Q flies.

EXAMPLE 4

25 This example describes characterization of several flies that contain a genetic element which suppresses polyglutamine toxicity. This example also describes the identification of dHDJ1, dTPR2 and dMLF that confer suppression of polyglutamine toxicity.

Characterization and Identification of dHDJ1

Of the 30 suppressor lines, EU3500 was selected for further studies. As shown by the scanning electron microscopy, the structural integrity of the eye of GMR-GAL4/UAS-127Q flies was dramatically improved in the presence of suppressor EU3500 (Figure 4C). The 5 eyes in flies carrying EU3500 retained their globular structure, and had a more uniform arrangement of bristles and pigmentation.

Internal eye structure was examined in horizontal cryostat sections of the heads. In un suppressed GMR-GAL4/UAS-127Q flies, immunolabeling of the HA-tagged polyglutamine peptides showed fluorescent aggregates. In contrast, although NIs appeared to 10 be the same in the presence of the EU3500 suppressor, the retinal structure was significantly improved (Figure 4C). Thus, the EU3500 suppressor was able to ameliorate the polyglutamine toxicity that occurred in the eye.

Plasmid rescue of the EU3500 suppressor P-element and its flanking genomic DNA and sequence analysis with a BDGP BLAST search identified an EST that matched the 15 genomic sequences starting 98 bp downstream of the P-element. This EST corresponded to at least 3 independent cDNA clones with different lengths of 3'UTR. The GH26396 clone (BDGP and Research Genetics, Inc.), a 1711 base pair cDNA sequence which encodes dHDJ1, a predicted protein of 334-amino-acid and molecular weight of 37 kDa, with an amino-terminal J domain and homologous to human Hsp40/HDJ1 was tested (54% identity 20 and 72% similarity using the parameters described above; Figure 6) (submitted directly to NCBI by Lee *et al.* (1995); Palter, K. *et al.* (1998);
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>).

In order to verify that the gene(s) immediately 3' to EU3500 was responsible for the observed suppression of polyglutamine toxicity, the corresponding cDNA, GH26396, 25 containing the coding sequences for dHDJ1, was placed in the transgenic vector pINDY6 (with UAS mediated expression) and microinjected into early stage fly embryos. In brief, the cDNA containing the coding region of dHDJ1 was removed from GH26396 (contained in the plasmid pOT2a, obtained from Research Genetics, Inc.) by complete digestion of 2.5 µg of plasmid DNA, in NEB #2 restriction enzyme buffer and 0.1 mg/ml BSA (New England 30 Biolabs), with 20 u HindIII for 3 hrs at 37°C to fragment pOT2a backbone, followed by partial digestion with 1, 2 or 4 u of PstI and XhoI for 10 min. at 37°C, and enzyme

inactivation at 65°C for 10 min. The reactions were run on 1% agarose gel and a 1816-bp-
fragment was isolated and purified by QIAquick gel extraction kit (Qiagen). This fragment,
which contains 106 bp PstI/EcoRI fragment of pOT2, 11 bp upstream of the reported 5'UTR,
the 5'UTR, *droJ1* ORF, 406 bp of the 579 bp reported 3'UTR, and a 23-bp-long poly(A), was
5 ligated into the transgenic vector pINDY6 PstI/XhoI site.

At least 3 independent transgenic lines carrying a heterozygous insertion of UAS-dHDJ1 together with GMR-GAL4/UAS-127Q closely reproduced the results of SEM, light microscopy, and immunofluorescence microscopy of cryostat sections observed for EU3500 P-element insertion (a representative line shown; Figure 4D). This result indicates that the
10 suppression of polyglutamine-dependent degeneration of the eye by the P-element insertion and its transgenic counterparts were due to increased levels of dHDJ1. Upon closer examination of the retinas, labeled with DAPI for staining of the nuclei and Y-11 anti-HA antibody/FITC for labeling 127Q peptides, in transgenic dHDJ1 flies expressing 127Q, cytoplasmic inclusions as well as nuclear ones were evident (Figure 4D).

15

Characterization and Identification of dTPR2

A second suppressor line, EU3220, was studied further. Although the improvement in eye morphology was less than EU3500, scanning electron microscopy revealed that this suppressor also significantly improved eye structure and pigmentation (Figure 4E). In
20 cryostat head sections, as with EU3500, EU 3220 improved retinal structure, although the effect was slightly weaker and the number of aggregates did not appear to change.

Plasmid rescue of the EU3220 suppressor P-element and its flanking genomic DNA and sequence analysis with a BDGP BLAST search identified an EST that matched the genomic sequences starting 293 base pairs downstream of the P-element. The corresponding
25 cDNA clone, GH09432 was sequenced. The P-element insertion was 649 bp 5' of the open reading frame (ORF) of a 2239-bp cDNA, corresponding to a predicted protein of 508 amino acids and molecular weight of 58 kDa, containing seven tetratricopeptide repeats and a C-terminal J domain. A protein database search revealed high homology (46% identity and 67% similarity using the parameters described above; Figure 7) between this and the human tetratricopeptide repeat protein 2 (TPR2). The identified drosophila sequences was therefore
30 designated dTPR2 (Figure 9).

At least 3 independent transgenic lines carrying a heterozygous insertion of UAS-dTPR2 together with GMR-GAL4/UAS-127Q confirmed that suppression by the EU3200 P-element and its transgenic counterpart were due to increased expression of dTPR2 (Figure 4F). This data indicates the EU3220 suppressor was also able to ameliorate the 5 polyglutamine toxicity that occurred in the eye.

Characterization and Identification of dMLF

A third suppressor line, EU2490 (the 2490th P-element insertion tested), dramatically counteracts the external eye and pigmentation defect caused by 127Q (Figure 5C). A lesser 10 internal improvement was seen in cryosections. P-element rescue was performed and the DNA flanking the 3' end of the P-element was sequenced (Pirrotta, Cloning Drosophila Genes: A Practical Approach, IRL Press, Oxford, Washington D.C., ed. D.B. Roberts, pp 83-110 (1986)). A BLAST search of the Berkeley *Drosophila* Genome Project (BDGP) server identified several ESTs with corresponding cDNA clones. A stretch of approximately 220 bp 15 of the genomic DNA, beginning at 385 bp downstream of the EU2490 P-element insertion site, was 97% identical to the DNA sequence beginning 54 bp downstream of a predicted ATG start site of an open reading frame (ORF). This ORF has been found in a cDNA clone, GH20101, from an adult head library. The ORF is 822 bp long and lies within a 1753-bp cDNA insert with 82 bp 5'UTR, 849 bp 3'UTR, and an 18-base polyA tail. The predicted 20 translation product of the ORF is a 273-amino-acid protein with a molecular weight of 30 kDa. Surprisingly, it is homologous to a human myeloid leukemia factor (MLF) (Yoneda-Kato *et al.*, *Oncogene*, 12:265-275 (1996)), with 32% identity and 49% similarity (Figure 8). Therefore, this gene is denoted as *Drosophila* myeloid leukemia factor, dMLF (Figure 10).

To confirm that the expression of dMLF is responsible for the suppression effect, the 25 cDNA insert in GH20101 was placed in the same kind of P-element vector as UAS-127Q, and transgenic lines established. Three independently established lines, each carrying a heterozygous autosomal insertion of UAS-dMLF in the presence of GMR-GAL4/UAS-127Q, reproduced the improvement in external eye structure and pigmentation to an even greater extent than did the original P-element insertion (Figure 5D and 5E). The internal eye 30 structure was only slightly improved; however higher doses of the suppressor gene almost completely restored both external and internal eye structures to normal (Figure 5F). Three

different transgenic lines were established, each carrying UAS-dMLF transgenic insertions on both the second and third chromosomes, and all exhibited greater improvements in eye structure. Nevertheless, as with the two previous suppressor genes described above, 5 fluorescent aggregates indicating the presence of polyglutamine nuclear inclusions were present in the eye. Thus, the suppressors do not appear to prevent aggregation of polyglutamine repeat sequence. Rather, they appear to enhance the ability of the cells to resist their toxic effect. This suggests that the suppressor genes identified act a later point along the pathway that results in cellular toxicity.

10 The protective effect of dMLF on polyglutamine toxicity in *Drosophila* neuronal tissues was ascertained. In brief, a neuron-specific driver, *Appl*, was used to drive expression of the GAL4 protein (*Appl*-GAL4); *Appl* is derived from the promoter region of the amyloid precursor protein-like gene, the *Drosophila* homologue of human amyloid precursor protein (APP) (Torroja *et al.*, *Current Biology*, **9**:489-492 (1999)). *Appl* is expressed exclusively in post-mitotic neurons of the central and peripheral nervous system, from mid to late stages of 15 embryogenesis onward (Martinmorris *et al.*, *Development*, **110**:185-195 (1990)).

Transgenic flies carrying only *Appl*-GAL4 developed normally. The same was true for three independent UAS-20Q insertions in the presence of *Appl*-GAL4. UAS-63Q, a UAS driven construct encoding a polyglutamine repeat sequence 63 residues in length however, had a strong toxic effect. In four transgenic lines tested three were pre-adult lethal; only one 20 gave rise to adults which were exclusively female. Since the *Appl*-GAL4 transgene was on the X chromosome, dosage compensation may have produced higher expression in males, resulting in the increased lethality. Three UAS-127Q lines were all pre-adult lethal in the presence of *Appl*-GAL4. Therefore, 63Q females were studied for suppression of toxicity by dMLF, using survival of adults versus age as a criterion.

25 The flies with *Appl*-GAL4 alone remained vital throughout the 20-day observation period; no polyglutamine aggregates were detected, as determined by anti-HA fluorescent staining, in brain or thoracic ganglion sections of the nervous system. In contrast, flies carrying *Appl*-GAL4 and a heterozygous insertion of UAS-63Q began to die by day 12 and almost all flies were dead by day 20. Shortly before death, the flies became progressively 30 lethargic, unable to climb the walls of the plastic vial; these were also counted as dead.

Cryosections of one-day-old adult *Appl*-GAL4/UAS-63Q flies revealed aggregates in the neuronal cell bodies of the cortices surrounding the neuropils of the brain and the thoracic ganglion. The fluorescent aggregates appeared to be almost exclusively localized to neuronal cell bodies, as evident by co-localization of the nuclear stain with staining by DAPI, and the absence of anti-HA stain in synaptic neuropil region. In plastic sections stained with toluidine blue, no signs of gross neuronal degeneration were observed, even in the last surviving flies at 20 days. Death may therefore be due to dysfunction of the neurons associated with polyglutamine repeat sequence expression.

Expression of dMLF with 63Q increased fly survival. At day 20, 60% of flies expressing dMLF with 63Q remained alive. Therefore, dMLF can protect against polyglutamine toxicity in neuronal tissues, as well as in the eye. These results also demonstrate that the eye can be used as a convenient morphological substitute in screening for suppression of polyglutamine toxicity in neuronal tissues.

15

EXAMPLE 5

This example describes several structural features characteristic of the dHDJ1, dTPR2 and dMLF that are likely to be important for the ability to decrease polyglutamine toxicity.

Both dHDJ1 and dTPR2 are implicated in protein chaperone function. For example, each has a J domain (Figure 6 and 7), a stretch of approximately 70 amino acids present in J proteins that stimulates ATPase activity of Hsp70 (Marsh *et al.*, *Hum. Mol. Genet.*, **9**:13-25 (2000)) which results in closure of its peptide-binding pocket, trapping protein substrates (Kazemi-Esfarjani *et al.*, *Science*, **287**:1837-1840 (2000)). J proteins also independently bind other proteins having secondary and tertiary structure (Ellis *et al.*, *Development*, **119**:855-865 (1993)).

25

Direct evidence for the role of heat shock proteins, particularly J proteins, in preventing protein aggregation has been provided *in vitro* by showing that a five-fold molar excess of *E. coli* DnaJ completely suppresses aggregation of a substrate protein (bovine mitochondrial rhodanese) (Freeman, *Cell*, **87**:651-660 (1996)). J proteins may also play a role in the proteasome degradation pathway, since the J domain of the simian virus 40 (SV40) large T antigen (TAg) was required for proteasome-dependent degradation of p130 (related to retinoblastoma tumor suppressor protein, pRB) in human osteosarcoma cell line

U-2 OS (Torroja *et al.*, *Current Biology*, **9**:489-492 (1999)). In fact, the J domains of human HDJ2 (also known as DNAJ2) or HSJ1 could substitute for the J domain in SV40 TAg, and substitution of a glutamine for a conserved histidine in the J domains could abolish that effect.

5 *Drosophila* TPR2 may also act as a suppressor in another way due to the presence of multiple TPR domains (Figure 7). TPR domains are made of 3 to 16 degenerate repeats of a 34-amino-acid stretch, each of which forms a pair of antiparallel α helices (Rorth, *Proc Natl Acad Sci USA*, **93**:12418-12422 (1996)). Multiple tandem TPR units assemble into right-handed superhelical structures that are suited for protein-protein interfaces. They are found
10 in proteins involved in various functions, including protein import, neurogenesis, stress response, and chaperone action (Warrick *et al.*, *Cell*, **93**:939-949 (1998); and Pirrotta, Cloning Drosophila Genes: A Practical Approach, IRL Press, Oxford, Washington D.C., ed. D.B. Roberts, pp 83-110 (1986)). The human TPR2 was isolated from a HeLa cell cDNA library in a two-hybrid screen, using as “bait” a 271-amino-acid fragment of GTPase-
15 activating protein-related domain (GRD) of neurofibromin, the neurofibromatosis type 1 (*NFI*) gene product (Warrick *et al.*, (1998), *supra*). Neurofibromin stimulates the GTPase-activity of p21 Ras and converts it from the active form (Ras-GTP) to its inactive form (Ras-GDP) (Yoneda-Kato *et al.*, *Oncogene*, **12**:265-275 (1996)). Conceivably, overexpression of dTPR2 in the fly eye inhibits the *Drosophila* homologue of neurofibromin (dNF1)
20 (Martinmorris *et al.*, *Development*, **110**:185-195 (1990)), by masking its GRD. This would increase the activity of Ras-GTP, which is known to inhibit the proapoptotic head involution defective (HID) protein Yoneda-Kato *et al.*, *Oncogene*, **18**:3716-3724 (1999)), and enhance the survival of eye cells.

In cultured cells transfected with full-length ataxin-1, or the androgen receptor, each
25 having expanded polyglutamines, co-expression of HDJ2/HSDJ resulted in 40-50% reduction in the number of cells containing aggregates (Ross *et al.*, *Blood*, **91**:4419-4426 (1998); and Sorensen *et al.*, *Cancer*, **86**:1342-1346 (1999)). Surprisingly, similar to the effect of *HSPA1L*, the EU3500 or EU3220 P-elements, or expression of their transgenic counterparts, inhibited deterioration of the eye structure, yet the formation of aggregates did not appear to
30 be suppressed. Since the GMR promoter acts early in eye development, it is possible that dHDJ1 and dTPR2 act at that early stage of differentiation, by binding to 127Q, maintaining

a non-toxic milieu, thus permitting eye development to proceed more normally.

Alternatively, these suppressor proteins, rather than directly interacting with 127Q peptide, may reduce its toxicity by a downstream effect.

The mechanism of protection against polyglutamine toxicity by dMLF may relate to the role of its human counterpart in cell survival and proliferation. In this regard, human MLF gene was first identified as a portion of a chimeric product including the nucleolar transport protein, nucleophosmin (NPM), in the chromosomal translocation t(3;5)(q25.1,q34) associated with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (Yoneda-Kato *et al.*, *Oncogene*, **12**:265-275 (1996)). In stable transfections of NIH3T3 mouse fibroblast cells with MLF cDNA, MLF Ab stained the cytoplasm, whereas the NPM-MLF chimeric product was exclusively nuclear and nucleolar (Yoneda-Kato *et al.*, *Oncogene*, **18**:3716-3724 (1999)). Neither MLF nor NPM alone had any detectable effect, but NPM-MLF induced apoptosis. The region necessary for apoptotic activity was narrowed down to a 92-amino acid stretch in MLF (Figure 8) (Yoneda-Kato *et al.* (1999), *supra*). Therefore, it is likely that the corresponding region of dMLF has a similar function.

When the anti-apoptotic protein Bcl-2 was expressed in the presence of NPM-MLF, the cells, instead of undergoing apoptosis, entered a proliferative phase. The induction of apoptosis resembles the anemia resulting from the cellular dysplasia in MDS patients, and the proliferative condition is reminiscent of the transformation of MDS to AML. Therefore, dMLF may protect against polyglutamine toxicity through its function as a component of cell survival signaling pathways. Accordingly, a fly genetic system that exhibits a dMLF phenotype, such as abnormal cell proliferation or a tumor, can be used to identify genes or other factors that have therapeutic value in treating myelodysplastic syndrome and acute myeloid leukemia in humans.

Another finding relating polyglutamine disease to cancer is the chromosomal translocation t(5;7)(q33;q11.2) observed in a patient suffering from chronic myelomonocytic leukemia (CMML), another form of MDS/AML (Ross *et al.*, *Blood*, **91**:4419-4426 (1998)). The putative chimeric product is made of Huntington-interacting protein 1 (HIP1) and platelet-derived growth factor β receptor. HIP1 was found in a yeast two-hybrid assay, using the NH₂-terminal portion of Huntington (encoded by the Huntington's disease gene, HD). Based on cell fractionation analyses and its similarity to Sla2p, a membrane-associated

protein in yeast, HIP1 appears to be involved in maintaining the integrity of the cell membrane (Kalchman *et al.*, *Nat. Genet.*, **16**:44-53 (1997); Sittler. *et al.*, *Mol. Cell*, **2**:427-436 (1998)). A lower incidence of cancer has been reported among individuals with Huntington's disease Sorensen *et al.*, *Cancer*, **86**:1342-1346 (1999)). Therefore, the 5 molecular pathways that give rise to Huntington's disease may be beneficial in preventing or treating cancer, and *vice versa*.

Discovery of dHDJ1, dTPR2 and dMLF as suppressors of polyglutamine toxicity underscores the fact that this fly system identifies genes effective in preventing one or more cellular or molecular aspects of polyglutamine diseases, without any knowledge of their 10 function. The sequence of the *Drosophila* genome was recently compiled (Adams *et al.*, *Science*, **287**:2185-2195 (2000)), and about 68% of known human cancer-associated proteins analyzed appear to have *Drosophila* homologues (Rubin *et al.*, *Science*, **287**:2204-2215 15 (2000)). However, dMLF was not among those listed. This may have been due to stringent criteria for homology, including a requirement for sharing a known protein domain, whereas MLF and dMLF both lack such domains.

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

20

WHAT IS CLAIMED IS:

1. A method of screening for genes that modulate polyglutamine toxicity comprising:
 - 5 (a) providing a first animal expressing a polyglutamine sequence, wherein the sequence produces polyglutamine toxicity in the animal;
 - (b) breeding the first animal to a second animal, wherein the second animal has a marker sequence inserted into its germline, thereby producing progeny;
 - (c) screening the progeny for increased or decreased polyglutamine toxicity relative to the first animal thereby identifying a progeny having increased or decreased polyglutamine toxicity; and
 - 10 (d) identifying one or more genes adjacent to or having an insertion of the marker sequence that confers increased or decreased polyglutamine toxicity in the progeny having increased or decreased polyglutamine toxicity.
- 15 2. The method of claim 1, further comprising step (e), identifying a mammalian homologue of the gene of claim 1.
3. The method of claim 2, wherein the mammalian homologue comprises a human homologue.
- 20 4. The method of claim 1, wherein the first and second animals are invertebrates.
5. The method of claim 4, wherein the invertebrates are of the genus *Drosophila melanogaster*.
- 25 6. The method of claim 1, wherein the marker sequence comprises a P element.
7. The method of claim 1, wherein the marker sequence comprises a polynucleotide sequence that disrupts or alters expression of one or more genes near the sequence.

8. The method of claim 1, wherein the marker sequence further comprises an expression control element conferring expression of the one or more genes near the marker.
9. The method of claim 8, wherein the expression control element increases or decreases expression of one or more of the near gene(s).
- 5
10. The method of claim 1, wherein the second animal is selected from a group of two or more animals having markers inserted into different locations of its genomic DNA.
- 10 11. The method of claim 10, wherein the second animal is selected from a group of 10 to 100, 100 to 500, or 500 or more of the animals.
12. The method of claim 1, wherein the second animal is selected from a library of animals having markers inserted at random locations of their genomic DNA.
- 15
13. The method of claim 12, wherein the library of animals is generated by random P element insertion.
14. The method of claim 1, wherein the polyglutamine sequence comprises a sequence having between about 35 to 50, or between about 50 to 100 glutamine residues.
- 20
15. The method of claim 1, wherein the polyglutamine sequence comprises a sequence having between about 100 to 150 glutamine residues.
- 25
16. The method of claim 1, wherein the polyglutamine sequence comprises a sequence having about 150 or more glutamine residues.
17. The method of claim 1, wherein the polyglutamine sequence further comprises a tag.
- 30 18. The method of claim 17, wherein the tag comprises an epitope tag.

19. The method of claim 18, wherein the epitope tag comprises a hemagglutinin sequence.
20. The method of claim 1, wherein the polyglutamine sequence is encoded by a polynucleotide containing a plurality of CAGs, CAAs or a combination thereof.
- 5
21. The method of claim 20, wherein expression of the plurality of CAGs, CAAs or combination thereof is conferred by a constitutive, regulatable or tissue specific expression control element.
- 10
22. The method of claim 21, wherein the regulatable element comprises an inducible or repressible element.
- 15
23. The method of claim 21, wherein the regulatable element comprises a GAL4 responsive sequence.
24. The method of claim 21, wherein the tissue specific element confers neural, retinal, muscle or mesoderm cell expression.
- 20
25. A progeny animal produced by the method of claim 1.
26. A transgenic animal comprising a transgene containing a plurality of CAG's and at least one CAA sequence encoding a polyglutamine repeat sequence.
- 25
27. The animal of claim 26, wherein the animal is an invertebrate.
28. The animal of claim 27, wherein the invertebrate animal is *Drosophila melanogaster*.
29. The animal of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 1:1 and 2:1.
- 30

30. The animal of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 2:1 and 5:1.
31. The animal of claim 26, wherein the number of CAG's to CAA's is in ratio of 5 between about 5:1 and 10:1.
32. The animal of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 10:1 and 50:1.
- 10 33. The animal of claim 26, wherein expression of the polyglutamine sequence is conferred by a constitutive, regulatable or tissue specific expression control element.
34. The animal of claim 33, wherein the tissue specific expression control element confers neural, retinal, muscle or mesoderm cell expression.
- 15 35. The animal of claim 33, wherein the tissue specific expression control element comprises an *App1* or *rhodopsin 1* promoter or GLASS transcription factor element.
36. The animal of claim 26, wherein the polyglutamine sequence is between about 30 and 20 50 amino acids in length.
37. The animal of claim 26, wherein the polyglutamine sequence is between about 50 and 65 100 amino acids in length.
- 25 38. The animal of claim 26, wherein the polyglutamine sequence is between about 100 and 150 amino acids in length.
39. The animal of claim 26, wherein the polyglutamine sequence is between about 50 and 80 200 amino acids in length.
- 30 40. The animal of claim 26, wherein the polyglutamine sequence further comprises a tag.

41. The animal of claim 26, wherein polyglutamine toxicity is produced in one or more tissue or organs of the animal.

5 42. The animal of claim 26, wherein the animal further comprises a marker sequence inserted into its genomic DNA, wherein the marker is located adjacent to a gene or inserted into a gene whose expression or activity increases or decreases polyglutamine toxicity in the animal.

10 43. The animal of claim 42, wherein the marker sequence is near or inserted into a gene containing a J domain.

44. The animal of claim 43, wherein the gene is HDJ1.

15 45. The animal of claim 43, wherein the gene is TPR2.

46. The animal of claim 43, wherein the marker sequence is near an MLF gene.

20 47. A method for identifying a compound that modulates polyglutamine toxicity in an animal comprising:
(a) contacting the animal of claim 41 with a test compound; and
(b) determining whether the test compound increases or decreases polyglutamine toxicity in the animal, where increased or decreased polyglutamine toxicity identifies the test compound as a compound that modulates polyglutamine toxicity.

25 48. The method of claim 47, wherein the compound is present in the animal's food or drink.

30 49. The method of claim 47, wherein the compound is administered to a tissue or organ of the animal.

50. A method of producing a transgenic animal characterized by polyglutamine toxicity comprising:
- (a) transforming an animal embryo or egg with a transgene comprising a plurality of CAA and CAG sequences encoding a polyglutamine sequence having a length sufficient to produce polyglutamine toxicity in the animal produced from the embryo or egg; and
- (b) selecting an animal that exhibits polyglutamine toxicity in one or more cells or tissues.

10

51. An isolated polynucleotide sequence having about 65% or more identity to a *Drosophila* TPR2 (dTTPR2) sequence set forth as SEQ. ID NO:2 and which encodes a polypeptide that decreases polyglutamine toxicity, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 11.

15

52. The polynucleotide sequence of claim 51, wherein the sequence encodes a subsequence of TPR2 that decreases polyglutamine toxicity.

20

53. The polynucleotide sequence of claim 51 operatively linked to an expression control element.

25

54. An isolated polynucleotide sequence that hybridizes under stringent conditions to a *Drosophila* TPR2 (dTTPR2) sequence set forth as SEQ. ID NO:2, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 11.

30

55. The polynucleotide sequence of claim 54, wherein the sequence comprises a polynucleotide having 20 or more contiguous nucleotides.
56. The polynucleotide sequence of claim 54, wherein the sequence comprises a polynucleotide having 50 or more contiguous nucleotides.

57. An isolated polynucleotide sequence having about 65% or more identity to a *Drosophila* MLF (dMLF) sequence set forth as SEQ. ID NO:4 and which encodes a polypeptide that decreases polyglutamine toxicity, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 12.
- 5
58. The polynucleotide sequence of claim 57, wherein the sequence encodes a subsequence of MLF that decreases polyglutamine toxicity.
- 10
59. The polynucleotide sequence of claim 57 operatively linked to an expression control element.
- 15
60. An isolated polynucleotide sequence that hybridizes under stringent conditions to a *Drosophila* MLF (dMLF) sequence set forth as SEQ. ID NO:4, with the proviso that the sequence is distinct from the EST sequences set forth in Figure 12.
61. The polynucleotide sequence of claim 60, wherein the sequence comprises a polynucleotide having 20 or more contiguous nucleotides.
- 20
62. The polynucleotide sequence of claim 60, wherein the sequence comprises a polynucleotide having 50 or more contiguous nucleotides.
63. A composition comprising a polynucleotide sequence encoding a human MLF polypeptide operatively linked to an expression control element in a pharmaceutically acceptable carrier.
- 25
64. A composition comprising a polynucleotide sequence encoding a human TPR2 polypeptide operatively linked to an expression control element in a pharmaceutically acceptable carrier.

65. A method of increasing survival of a cell having polyglutamine toxicity, comprising contacting the cell with an amount of TPR2 or MLF polypeptide sequence or a polynucleotide sequence TPR2 or MLF polypeptide to increase survival of the cell.
- 5 66. A method of decreasing apoptosis of a cell, comprising contacting the cell with an amount of TPR2 or MLF polypeptide sequence or a polynucleotide sequence TPR2 or MLF polypeptide to decrease apoptosis of the cell.
- 10 67. A method of decreasing polyglutamine toxicity in a cell having or susceptible to polyglutamine toxicity, comprising contacting the cell with an amount of J domain containing polypeptide, TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide sequence to decrease polyglutamine toxicity in the cell.
- 15 68. The method of claim 67, wherein the cell is a neural, retinal, muscle or mesoderm cell.
- 20 69. The method of claim 67, wherein the toxicity is decreased by decreasing cell death or increasing cell survival.
- 25 70. A method of decreasing polyglutamine toxicity in a tissue or organ of a subject having or at risk polyglutamine toxicity, comprising contacting the tissue or organ with an amount of a J domain containing polypeptide, a TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, to decrease polyglutamine toxicity in the tissue or organ of the subject.
71. The method of claim 70, wherein the tissue is brain, eye, muscle or mesoderm.
- 30 72. A method of decreasing the severity of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in a subject having or at risk

of a frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder, comprising administering to the subject an amount of J domain containing polypeptide, a TPR2 or MLF polypeptide sequence, or a polynucleotide sequence encoding the J domain containing polypeptide, TPR2 or MLF polypeptide, 5 to decease the severity of the frontotemporal dementia, prion disease, polyglutamine disorder or protein aggregation disorder in the subject.

73. The method of claim 72, wherein the method comprises prophylactic administration.
- 10 74. The method of claim 72, wherein the disorder is a neurological or muscle disorder.
75. The method of claim 72, wherein the disorder impairs long term or short term memory or coordination of the subject.
- 15 76. The method of claim 72, wherein the disorder is characterized by the presence of protein aggregates, amyloid plaques, degeneration or atrophy in an affected tissue or organ.
77. The method of claim 72, wherein the disorder is selected from the group consisting of Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jacob's disease (CJD), bovine spongiform encephalopathy, Huntington's disease (HD), Machado-Joseph disease (MJD), Spinocerebellar ataxias (SCA), dentatorubropallidoluysian atrophy (DRPLA), Kennedy's disease, stroke and head trauma.
- 20
- 25 78. The method of claim 72, wherein the severity is decreased by decreasing cell death or increasing cell survival.
79. The method of claim 72, wherein the severity is decreased by decreasing protein aggregation.

ABSTRACT

The present invention is based on an *in vivo* animal model that mimics human cellular and tissue degenerative disorders. The animal model exhibits cellular toxicity in response to 5 expanded polyglutamine repeat sequences. The animal model is therefore useful for identifying genes or other compounds that modulate cellular and tissue degeneration and cell survival, for example, in neural, muscle, mesoderm, kidney and other tissues associated with frontotemporal dementia, prion diseases, polyglutamine disorders and protein aggregation disorders. Genes that suppress degeneration identified using the animal model include HDJ1, 10 TPR2 and MLF. These genes, and their human homologues, functional fragments and probes are therefore useful in treating such disorders and for diagnostic purposes. Accordingly, methods for identifying nucleic acids and other compounds that modulate frontotemporal dementia, prion diseases, polyglutamine disorders and protein aggregation 15 disorders is therefore provided. Pharmaceutical compositions comprising HDJ1, TPR2 and MLF genes, and subsequences encoding functional polypeptides are also provided, as they are useful in treating such degenerative disorders.

FIGURE 1A

20CAGHA

CTGCAGGCCAGCGTCTGATAAGTGAATTGCCGCCACATGGGAGGCCAC
CGTCAACCCCCCAGCAGCAGCAACAGCAGCAGCAACAGCAACAGCAGCAGC
AACAACAGCAGCAGCAACAGACTAGTCGTACGTATCCCTATGACGTGCCGA
CTATGCGTAG

127CAGHA

CTGCAGGCCAGCGTCTGATAAGTGAATTGCCGCCACATGGGAGGCCAC
CGTCAACCCCCCAGCAGCAGCAACAGCAGCAGCAACAGCAACAGCAGCAGC
AACAACAGCAGCAGCAACAGCAACAGCAGCAGCAACAGCAGCAGCAGCAGC
AGCAACAGCAGCAGCAACAGCAGCAGCAACAGCAACAGCAGCAGCAGCAGC
AGCAGCAGCAACAGCAGCAGCAACAGCAACAGCAGCAGCAGCAGCAGCAGC
AGCAGCAGCAACAGCAGCAGCAACAGCAACAGCAGCAGCAGCAGCAGCAGC
AGCAACAGCAACAGCAGCAGCAACAGCAGCAGCAACAGCAGCAGCAGCAGC
AGCAACAGCAGCAGCAACAGCAACAGCAGCAGCAACAGCAGCAGCTGCAAC
AGCAACAGCAGCAGCAACAGCAACAGCAGCAGCAACAGCAGCAGCAGCAGCAGC
CCTATGACGTGCCGACTATGCGTAG

FIGURE 1B

20QHA

MGGPPSTPQ₂₀*T*SRTYPYDVPDYA

127QHA

MGGPPSTPQ₁₂₇*T*SRTYPYDVPDYA

Figure 1. A) DNA sequences of 20QHA and 127QHA and B) their predicted protein sequences. The protein-coding region is underlined. The Kozak sequence is in italic.

FIGURE 2

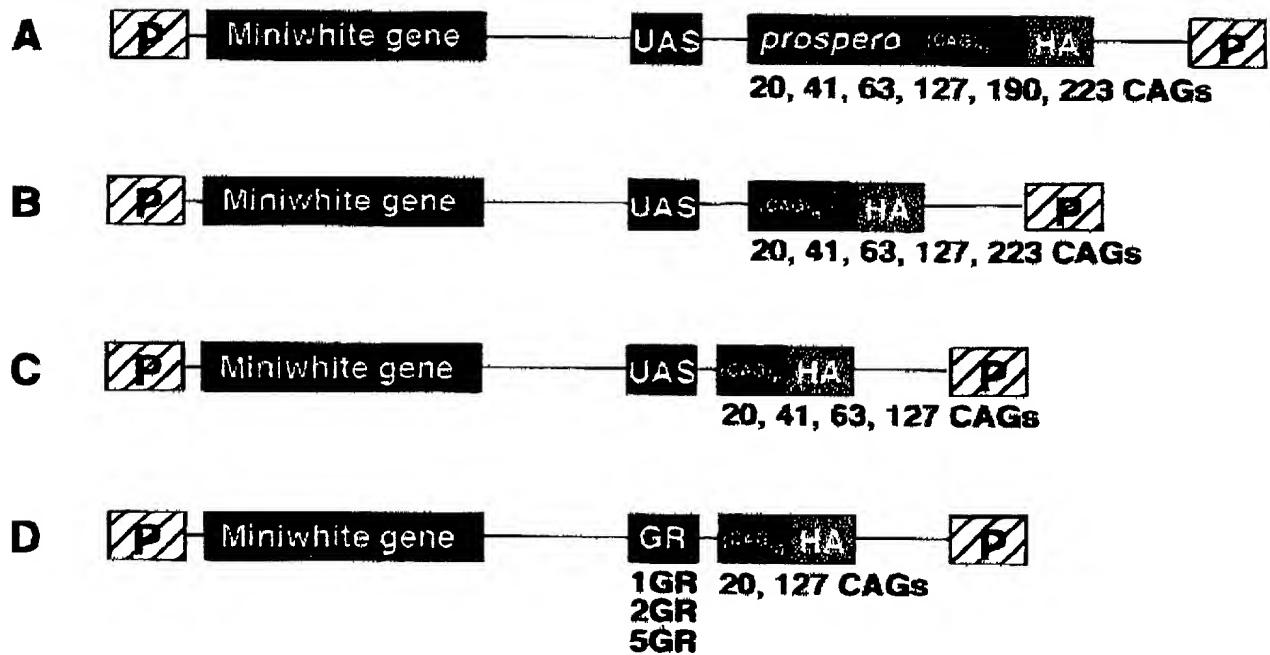


Figure 1. P-element plasmid constructs for production of transgenic flies. Each construct has two P-elements for chromosomal insertion. To facilitate identification of transformed flies, a miniwhite gene is included to produce red pigmentation in the eye. **A)** Plasmids carrying the full-length cDNA encoding the fly PROSPERO with various CAG repeat sizes. The expression of PROSPERO is regulated by five tandem upstream activating sequences (UAS). The yeast transcription factor GAL4 activates the transcription from these UAS elements. At its 3'-end, *prospero* cDNA is joined, in-frame, to a short DNA sequence that codes for a heterologous epitope, hemeagglutinin (HA). Antibodies against HA will be used to label the protein in immunohistochemical assays and Western blots. **B)** Plasmids carrying a partial cDNA encoding 422 amino acids of the C-terminal end of PROSPERO with various CAG repeat sizes. **C)** Plasmids carrying a DNA sequence that only encodes polyglutamines of various sizes. **D)** Plasmids carrying a DNA sequence that only encodes polyglutamines of various sizes, expressed under the control of one, two or five GLASS response elements (1GR, 2GR, or 5GR). The eye-specific protein GLASS activates the expression of polyglutamines from the GLASS response elements.

FIGURE 3

Generation of the P-element insertion and screening for modifiers

M P[Δ 2-3]/P[Δ 2-3] X F EP55/EP55

↓

M EP55/Y;; P[Δ 2-3]/+ X F w/w

↓

M w/Y;pEP/+;+ or w/Y;+;pEP/+ X F w;GMR/CyO;127Q/127Q

↓

Progeny screened for eye phenotype

Isolation of the new P-element insertion (pEP = suppressor or enhancer)

M (GMR;127Q)/pEP X F (CyO;TM3)/Xa

↓

M GMR/CyO;pEP/TM3 X F w1118

↓

M GMR;TM3 or CyO;pEP X F w;GMR/CyO;127Q/127Q to test

X F (CyO;TM3)/Xa to establish line

↓

M +/CyO;pEP/TM3 X F +/CyO;pEP/TM3

↓

pEP/TM3 or pEP/pEP established lines

Genetic scheme used for generating P-element mutants, screening for modifiers of polyglutamine toxicity, and isolating a hypothetical modifier P-element insertion on chromosome 3. Homozygous EP55 virgin females were crossed with males homozygous for a defective transposon, expressing the transposase. The F1 male progeny were crossed with virgin w1118 females. The F2 Male progeny that had coloured eyes and lacked the transposon's genetic markers were selected, as they contain a new stable insertion on an autosomal chromosome. These males were crossed with flies heterozygous for GMR-GAL4 on chromosome 2, balanced by CyO chromosome, and homozygous for UAS-127Q on chromosome 3. The resulting F3 progeny were screened for eye phenotype. Once a modifier was found, a single male was crossed to female (CyO;TM3)/Xa. The resulting male progeny were crossed to w1118 flies to separate the P-elements. This resulted in colored-eye progeny that carry a balancer for one chromosome and a P-element on another. Males from such progeny were tested for modifier activity by crossing to female w;GMR/CyO;127Q/127Q. The lines were established by crossing the latter males to (CyO;TM3)/Xa, and by crossing the resulting flies carrying CyO and TM3 balancers. EP55: source of transposable P-element; P[Δ 2-3]: source of transposase; F: female; M: male; CyO: balancer chromosome 2; TM3: balancer chromosome 3. Xa: translocation (2;3) Xa. (Chromosome 4 is omitted.)

FIGURE 4

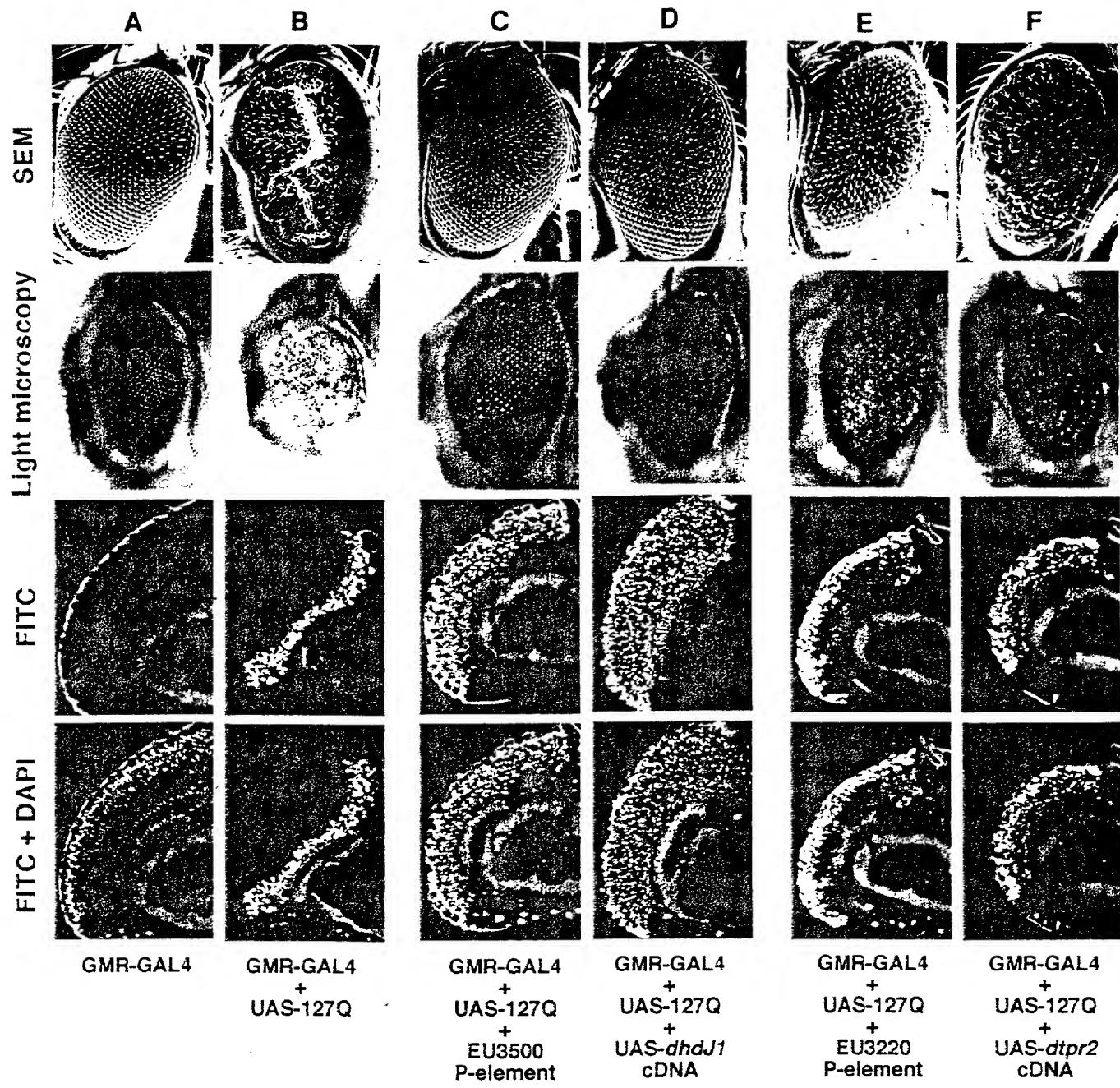


FIGURE 5

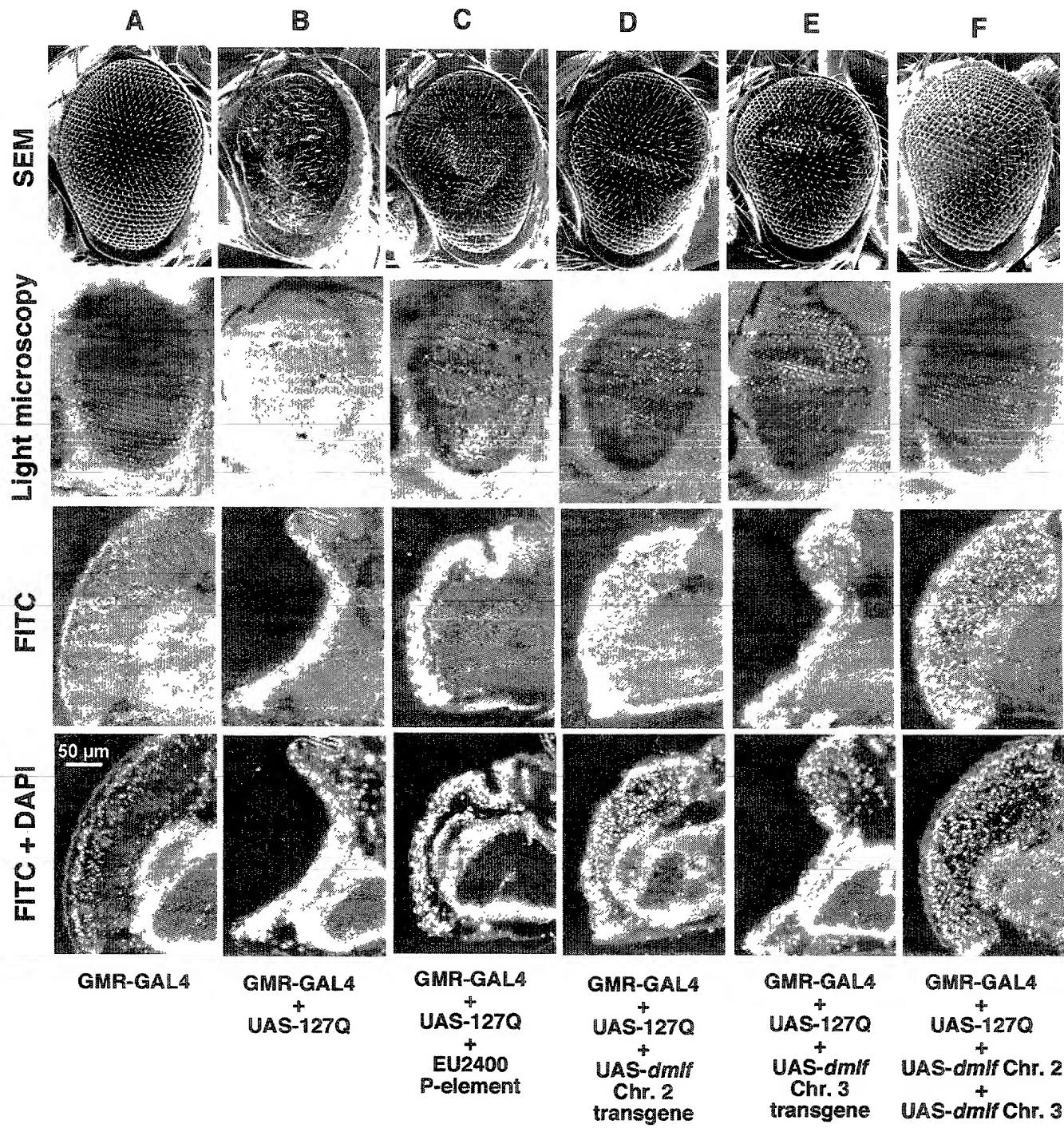


FIGURE 6

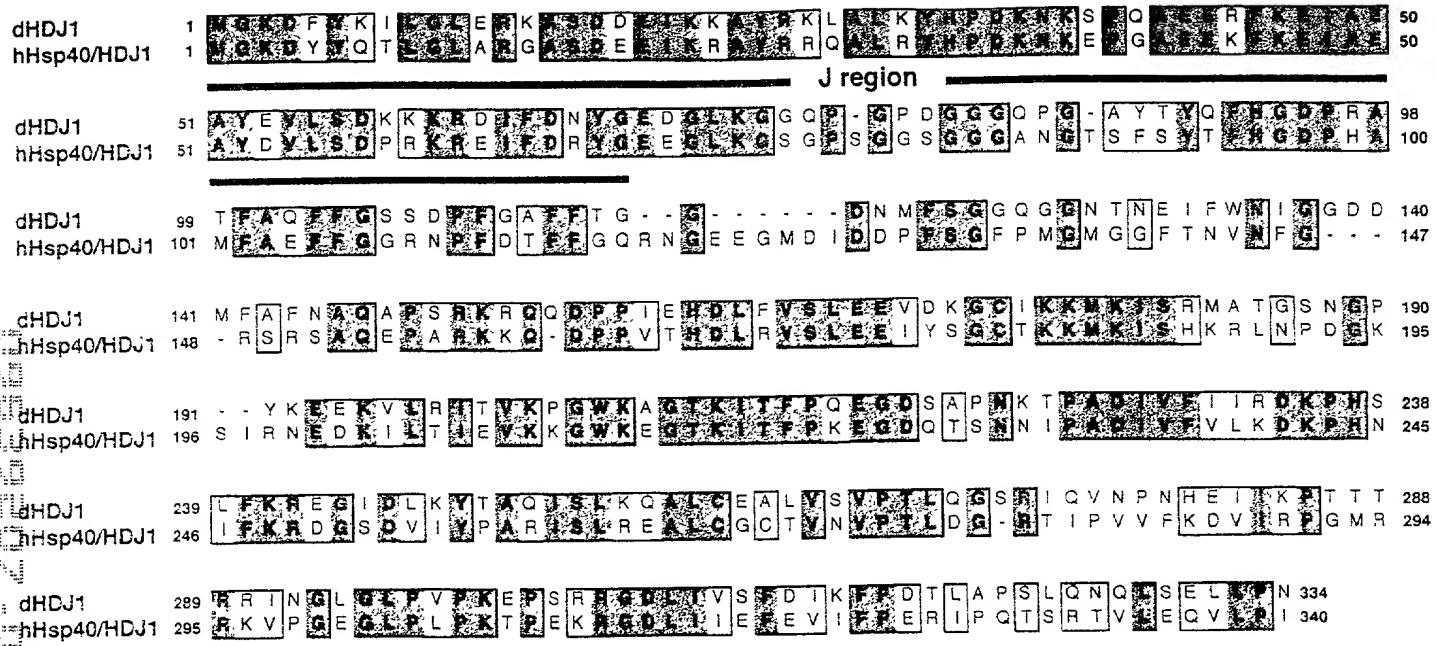


FIGURE 7

Sequence alignment of dTPR2 and hTPR2:

- N-terminal:** dTPR2 1 - 40, hTPR2 1 - 40
- TPR:** dTPR2 51 - 100, hTPR2 24 - 69
- J region:** dTPR2 101 - 150, hTPR2 70 - 119
- TPR:** dTPR2 151 - 200, hTPR2 120 - 169
- C-terminal:** dTPR2 201 - 250, hTPR2 170 - 219
- TPR:** dTPR2 251 - 300, hTPR2 220 - 269
- J region:** dTPR2 301 - 350, hTPR2 270 - 319
- TPR:** dTPR2 351 - 400, hTPR2 320 - 369
- C-terminal:** dTPR2 401 - 450, hTPR2 370 - 419
- J region:** dTPR2 451 - 498, hTPR2 420 - 469
- C-terminal:** dTPR2 499 - 508, hTPR2 470 - 484

FIGURE 8

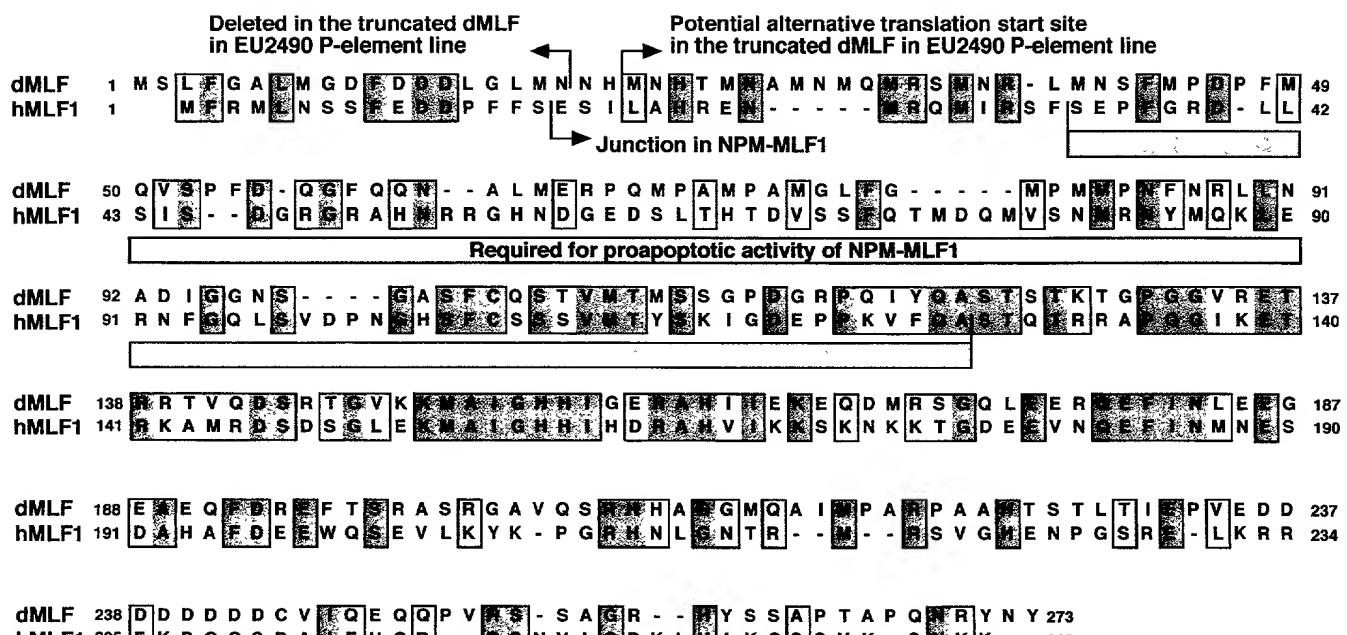


FIGURE 9A

dTPR2 Protein 508 amino acids

MDDEVIEISDSEREETSSNSEMDVEITTEQPTIDVKAEQIVPKDAATIAEEKKLG
NDQYKAQNYQNALKLYTDAISLCPDSAAYYGNRAACYMMLNNYNSALTDARH
AIRIDPGFEKAYVRVAKCCLALGDIIGTEQAVKMVNELNSLSTA VAAEQTAAQK
LRQLEATIQANYDTKS YRN VVFYLD SALKLAPACLKYRLLKAEC LAFLGRCDEA
LDIAV SVMKLD TTSADAIYVRGLCLYYTDNL DKGILHFERALTLD PDHY KSKQM
RSKCKQLKEMKENG NMLFKSGRYREAHVIY TDALKIDEHNKDINSKL LYNRALV
NTRIGNLREAVADC NR VLE LNSQ YLKALL RAR CYNDL EKFEESVAD YET ALQL
EKTPEIKRMLREAKFALKKSKRKDYYKILGIGRNASDDEIKKAYRK KALV HPD
RHANSSAEERKEEELKFKEVGEAYAILS DAHKSRYDSG QDIEEQEQAD FDPNQ
MFRTFFQFNGGGRNNSSFNEF

FIGURE 9B

dTPR2 cDNA 2239 base pairs

GGCACGAGCCACTACTTCGCATGGCACGCTTTCCGTGTGCTCGGTTCGTT
CGGCCATACAAAACACAAAATTCAAGTTAAA ACTAAATAGGCAACTAAA
GGGAAGCCGAGCGAATAAAGT GATTGCTGAAAGAGACGTAAGAAAGTTA
ATCGCATCGAAGGCACCAGAAATCGGGGATTCTAACACGGCGCGTGC
CGTACATACATACGCAAGCGCACACACACGAACAATTACTGCCATTGAC
GCAAAAGCGAAAAAGCAGT GGAATAAAGGGGATTGACAAATAACAACGTT
TTGCAAGCACTGGACTCTGGTCGCTGGTCTTCATTTGTATTGCCACG
CATGGACGACGAAGTAATTGAAATTAGCGACAGCGAACCGAAGAAACCTC
ATCGAACTCCGAAATGGATGTGGAATAACGACAGAACAGCAACC ATCGAT
GTCAAAGCAGAGCAAATTGTGCCAAGGACCGCGAACATTGCCAGGAG
AAGAAGAAACTGGCAACGACCAATACAAGGCAGAACTATCAGAATGCA
CTCAAGCTCTACACGGATGCCATATCGCTGTGTCCGGACTCGCGGCATA
TGGCAATCGGGCCGCTGCTACATGATGCTGCTCAACTATAATAGGCCCTG
ACCGACGCCGACACGCCATACGCATCGATCCGGCTCGAGAACGGCTACG
TCCGTGGCCAAGT GCTGTCTGGCCCTGGCGACATTATGGCACCGAAC
GGCGTCAAAATGGTCAACGAGCTGAATTGCTTAGCACGGCTGTGCTGCC
GAACAGACGGCGCGCAAAAGT GCGCCAATTGGAGGCCACCATTAGCG
AACTACGATACGAAAT CCTATCGCAATGTGGCTTCTATTGGATAGTGC
GAAATTGGCGCCCGCTGTTGAAATATCGTCTACTCAAGGCTGAGTGC
CATTGGGGCGATGTGATGAGGCCTGGACATTGCGGTCA GTGTAATGAA
ACTGGATACCACATCGCGGATGCGATATCGT GAGAGGTCTGTGC
TACACGGACAACCTGGACAAGGGAAATTCTCATTGAGCGCCCTGACCC
TCGACCCGGACCACTACAAGTCCAAGCAGATGCGCAGCAAATGCAAGCAGCT
CAAGGAGATGAAGGAGAACGGCAATATGCTATTCAAGT CGGGT CGGTATCGC
GAGGCACACGTATCTACACGGACGCCCTGAAGATCGATGAACACACAAGG
ATATCAATTGAAATTGCTTACAATCGGGCTTGTCACACCGCTATTGGC
AATTGCGAGAGGCCGTGGCGATTGCAATCGAGTGCTGGAGCTGAATAGTC
AGTATCTGAAGGCTCTGGTCTGCGAGCGCGCTGCTACAATGATCTGGAGAA
GTTCGAGGAGTCGGTGGCGACTATGAGACGGCGCTGCAGCTGGAGAAGAC
GCCGGAGATTAAGCGAATGCTGCGCGAGGCCAAGTTGCGITGAAGAAGTCG
AAGCGAAAGGACTACTACAAGATCCTGGCATTGGACGCAATCGCTCCGACG
ACGAGATCAAGAAGCGTATCGCAAAAAGGCCTGGTACATCATCGGATCG

FIGURE 9B (Continued)

ACACGCAAACAGCAGTGCCGAGGAGCGCAAGGAGGAGGAGCTCAAGTTCAA
GGAGGTGGCGAGGCCTACGCCATACTGTCGGATGCTCACAGAAGTCGCGC
TACGACAGCGGCCAGGATATCGAGGAGCAGGAGCAAGCCGACTTCGATCCG
AATCAAATGTTCCGCACATTCTCCAATTCAACGGCGGTGGCCGGAATAATT
ATCGTTCAACTTGAGTTCTAGGATCCAACGAGTGTGTTACCACACAGA
GAAGAAAGACCATCTCAATCCCATACTTCTGCCTCATCCGAAACCAACATAC
AGCAGCGCACAAATTGAACTCTTTACATATTCTTTCCAAAAAGCAAGA
AAATACCACATTGATTATGTTAACGAATGAATATATGCCAAGTTATTGAA
AAAATATTCTAAATCAAATAATGCAACTAAATTCCAGTGTAAAGTTCACATT
TTTAAATGTTCTTCTGGATTTTTTCGGCAACATTAATAAAATCATGGGAG
ATTGTGTTAAATAAACAGAAATACATATAAAAAAAAAAAAAAAA

FIGURE 10A

dMLF Protein 273 amino acids

MSLFGALMGDFDDDLGLMNNHMNHTMNAMNMQMRSMNRLMNSFMPDPFMQ
VSPFDQGFQQNALMERPQMPAMPAMGLFGMPMMPNFNRLLNADIGGNSASF
CQSTVMTMSSGPDGRPQIYQASTSTKTGPGVRETRRTVQDSRTGVKKMAIGHH
IGERAHIIKEQDMRSGQLEERQEFinLeeGEAEQFDREFTSRASRGAVQSRHHAG
GMQAIMPARPAAHTSTLTIEPVEDDDDDDDCVIQEQQPVRSSAGRHYSSAPTAP
QRNYN

FIGURE 10B

dMLF cDNA 1753 base pairs

GGCACGAGGAAAATTCGTAAAATTCTGCATACGGAAAGAAGAAAATTCA
GAGCAACAGAAAGCCAACACAATCCACAAAAATGTCTTATTGGAGCGTTG
ATGGGTGATTTCGACGACGATCTCGGCCATTGAACACCACATGAACCACA
CTATGAACCGCATGAACATGCAGATGCCTCGATGAATGCCCTGATGAACAG
CTTATGCCGATCCCTCATGCAGGTCTGCCCTTGACCAGGGATTCCAGC
AGAACGCTCTCATGGAGCGTCCGCAGATGCCGGCATGCCAGCATGGCCT
CTTCGGCATGCCATGATGCCAAACTTAATGCCCTGTTGAACGCTGATATTG
GTGGCAATTAGCGCATCCTCTGCCAGAGCACCCTGATGACCATGTCATC
GGGTCCGATGGCGTCCAGATCTACCAGGCCAGCACTAGTACCAAAACA
GGACCGGGAGGCCTGAGACCCGCAGGACGGTGCAGGACTCGCGCACT
GGGTGAAGAAGATGCCATTGGTCATCACATGGCGAGCGGGCACACATT
TTGAGAAAGAGCAGGACATGCGCTCAGGACAACGGAGGAGCGCCAGGAGT
TCATTAATCTGGAGGAGGGAGAAGCCGAGCAGTTGACAGGGAGTTACATC
GCGCGTAGTCGGAGCGGTGCAGTCAGACATCATGCTGGTGGCATGCAG
GCCATCATGCCGCCGTCCAGGCCACACACCTGACGTTGACCATTGAGC
CAGTGGAGGACGACGACGATGATGATGACTGTGTAATCCAGGAGCAGC
AACCGGTTCGCTCCGCCATTATTCCAGTGCACGCCAGGGCACC
GCAGAACAGATATAATTACTAAATCTAAAGTCATAACAGTATATTACTAA
CTATCCGATAAAACAGAAACAGAATTGCATACTATAAATTCTGCTAATTAC
ATTCCAACCTCGTTCAAACGAAACGAATATCGAATCGAAATCATAGAATGC
ACAGAGCAGCATACTCCACATCCCTATGCCGCCATCCGAGGCGCCAACAA
CGTCCGTAAAACATTTCACACGGAGGACGAAGCGGCCAGCTCTACAAGG
CGGTCAGCGGGCAAGAAGAAGTAGTAGAACGTGATCATCTGTATGCCAA
CATCTCCGCATCGCACACTCAAAACACTAGGAAGCAAAGCGTTGGTTCT
GTTCCATAGCAGGAAAACCAATTCAAATATTAAACAAACACAATTCTTA
CCAGTTCTGTCTTATCCTCGGTGAGTCGACCGAGAATGCAACACTAAAAATGT
ACAACCTCAAGATGCTATTGATGTGCACGCAGGATAACAGAACACTGCTTA
AATTACTAAAACAAATGTGACTATTCAACGCCAAATCATTACAACACAC
ACTCTCAGACCTAATCGAAAATTCAATGAAAGTAATGGAATATATATGAAA
TCGTAATTATAAGTTGAATTATTGATTAAATTCTCAAGTTTAGATTGTT
AGCCACTAAGCTTAAATTATGGATGCCAGTTAGCGTGCACGCCAAATGAACACAAT
TGATTGAAGGCTCCGAACGATAGAAAACAACAATTACCAATTCCCCAAATA
CATGTAATTGTAAGGCCTAAGTAAATTGTTAACGTGAATTAAATTAAATGGTA
ATTACATTATAATAGAAAAAAAAAAAAAA

FIGURE 11A

ClustalW Formatted Alignments

FIGURE 11A (Continued)

FIGURE 11A (Continued)

FIGURE 11A (Continued)

AA950779	570	G C C A T A T C G C T	541
A1456556	574		545
A1457049	566		545
A1110008	577	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
AA201154	580		545
AA952015	577	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
A1516974	586	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
AA201429	587		545
AA392620	585		545
AA439655	586	G C C A T A T C G C T G T G	545
A1544064	580	G C C A T A T C G C T G T G C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
A1516760	582		545
AA978757	480		545
AA202799	368		545
AA735495	504		545
AA201527	371		545
AA438619	378		545
AA1516274	589	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
AA440018	583	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
AA820977	589	G C C A T A T C G C T G T G T C C G G A	545
A1455565	586		545
dTPR2 (GH09432) GenBank submitt	588	G C C A T A T C G C T G T G T C C G G A C T C G G C G G C A T A C T A T G G C A A T C G G G C C G	545
 AA950779	581		
A1456556	574		
A1457049	566		
A1110008	626	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
AA201154	580		
AA952015	627	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
A1516974	635	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
AA201429	367		
AA392620	585		
AA439655	570		
A1544064	610	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
A1516760	582		
AA978757	480		
AA202799	368		
AA735495	504		
AA201527	371		
AA438619	378		
AA1516274	519	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
AA440018	513	C T G C T A C A T G A T G C T G C T C A A C T	545
AA820977	589		
A1455565	586		
dTPR2 (GH09432) GenBank submitt	588	C T G C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C	545
 AA950779	581		
A1456556	574		
A1457049	566		
A1110008	676	A C G	545
AA201154	580		
AA952015	577	A C G C C A T A C G C A T C G A T C C G G G C T T C G A G A A G G C C T A C G T C C G T G T G G C C	545
A1516974	678		
AA201429	387		
AA392620	585		
AA439655	570		
A1544064	680	A C G C C A T A C G C A T C G A T C C G G G C T T C G A G	545
A1516760	582		
AA978757	480		
AA202799	368		
AA735495	504		
AA201527	371		
AA438619	378		
AA1516274	689	A C G C C A T A C G C A T C G A T C C G G G C	545
AA440018	636		
AA820977	589		
A1455565	586		
dTPR2 (GH09432) GenBank submitt	688	A C G C C A T A C G C A T C G A T C C G G G C T T C G A G A A G G C C T A C G T C C G T G T G G C C	545
 AA950779	581		
A1456556	574		
A1457049	566		
A1110008	679		
AA201154	580		
AA952015	677	A A G T G C T G T C T G G C C C T G G G C G A C A T T A T T G G C C C G A A C A G G C C G T C A A A	545
A1516974	678		
AA201429	387		
AA392620	585		
AA439655	570		
A1544064	689		
A1516760	582		
AA978757	480		
AA202799	368		
AA735495	504		
AA201527	371		
AA438619	378		
AA1516274	682	A A G T G C T G T C T G G C C C T G G G C G A C A T T A T T G G C A C C G A A C A G G C C G T C A A	545
AA440018	636		
AA820977	589		
A1455565	586		
dTPR2 (GH09432) GenBank submitt	738	A A G T G C T G T C T G G C C C T G G G C G A C A T T A T T G G C A C C G A A C A G G C C G T C A A	545

FIGURE 11A (Continued)

AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	777 A T G G T T	782
AI516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	599	588
AI455565	596	565
dTPR2 (GH09432) GenBank submit	788 A A T G G T C A A C G A G C T G A A T T C G C T T A G C A C G G C T G T T G C T G C C G A A C A G A	837
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	599	588
AI455565	596	565
dTPR2 (GH09432) GenBank submit	888 C G G C G G C G C A A A A G T T G C G C C A A T T G G A G G C C A C C A T T C A G G C G A A C T A C	887
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	599	588
AI455565	596	565
dTPR2 (GH09432) GenBank submit	888 G A T A C G A A A T C C T A T C G C A A T G T G G T C T T C T A T T T G G A T A G T G C C T T G A A	937
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	599	588
AI455565	596	565
dTPR2 (GH09432) GenBank submit	938 A T T G G C G C C C G C C T G T T G A A A T A T C G T C T A C T C A A G G C T G A G T G C C T T G	987

FIGURE 11A (Continued)

AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	692	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	988	C A T T T T T G G G G C G A T G T G A T G A G G C C T T G G A C A T T G C G G T C A G T G T A A T G 1037
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	692	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1038	A A A C T T G G A T A C C A C A T C G G C G G A T G C G A T A T A C G T G A G A G G T C T G T G C C T 1087
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	692	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1088	G T A C T A C A C G G A C A A C C T G G A C A A G G G A A T T C T T C A T T T C G A G C G C G C C C 1137
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	692	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1138	T G A C C C C T C G A C C C G G A C C A C T A C A A G T C C A A G C A G A T G C G C A G C A A A T G C 1187

FIGURE 11A (Continued)

AA950779	581		580
AI456556	274		273
AI457049	586		585
AI110008	679		678
AA201154	530		529
AA952015	783		782
AI516974	678		677
AA201429	387		386
AA392620	535		534
AA439655	570		569
AI544064	689		688
AI516760	532		531
AA978757	480		479
AA202799	368		367
AA735495	504		503
AA201527	371		370
AA438619	378		377
AI516274	692		691
AA440018	636		635
AA820977	589		588
AI455565	586		585
dTPR2 (GH09432) GenBank submitt	1188	A A G C A G C T C A A G G A G A T G A A G G A G A C G G C A A T A T G C T A T T C A A G T C G G G	1287
AA950779	581		580
AI456556	274		273
AI457049	586		585
AI110008	679		678
AA201154	530		529
AA952015	783		782
AI516974	678		677
AA201429	387		386
AA392620	535		534
AA439655	570		569
AI544064	689		688
AI516760	532		531
AA978757	480		479
AA202799	368		367
AA735495	504		503
AA201527	371		370
AA438619	378		377
AI516274	692		691
AA440018	636		635
AA820977	589		588
AI455565	586		585
dTPR2 (GH09432) GenBank submitt	1288	T C G G T A T C G C G A G G C A C A C G T T A T C T A C A C G G A C G C C C T G A A G A T C G A T G	1287
AA950779	581		580
AI456556	274		273
AI457049	586		585
AI110008	679		678
AA201154	530		529
AA952015	783		782
AI516974	678		677
AA201429	387		386
AA392620	535		534
AA439655	570		569
AI544064	689		688
AI516760	532		531
AA978757	480		479
AA202799	368		367
AA735495	504		503
AA201527	371		370
AA438619	378		377
AI516274	692		691
AA440018	636		635
AA820977	589		588
AI455565	586		585
dTPR2 (GH09432) GenBank submitt	1289	A A C A C A A C A A G G A T A T C A A T T C G A A A T T G C T T T A C A A T C G G G C T T G G T C	1307
AA950779	581		580
AI456556	274		273
AI457049	586		585
AI110008	679		678
AA201154	530		529
AA952015	783		782
AI516974	678		677
AA201429	387		386
AA392620	535		534
AA439655	570		569
AI544064	689		688
AI516760	532		531
AA978757	480		479
AA202799	368		367
AA735495	504		503
AA201527	371		370
AA438619	378		377
AI516274	692		691
AA440018	636		635
AA820977	589		588
AI455565	586		585
dTPR2 (GH09432) GenBank submitt	1308	A A C A C G C G T A T T G G C A A T T T G C G A G A G G C C G T G G C C G A T T G C A A T C G A G T	1327

FIGURE 11A (Continued)

AA950779	581	
A1456556	274	
A1457049	566	
A1110008	679	
AA201154	530	
AA952015	783	
A1516974	678	
AA201429	367	
AA392620	535	
AA439655	570	
A1544064	689	
A1516760	532	
AA978757	480	
AA202799	368	
AA735495	504	
AA201527	371	
AA438619	378	
A1516274	692	
AA440018	636	
AA820977	599	
A1455565	566	
dTPR2 (GH09432) GenBank submitt	1388	G C T G G A G C T G A A T A G T C A G T A T C T G A A G G C T C T G T T G C T G C G A G C G C G C T
		1437
AA950779	581	
A1456556	274	
A1457049	566	
A1110008	679	
AA201154	530	
AA952015	783	
A1516974	678	
AA201429	367	
AA392620	535	
AA439655	570	
A1544064	689	
A1516760	532	
AA978757	480	
AA202799	368	
AA735495	504	
AA201527	371	
AA438619	378	
A1516274	692	
AA440018	636	
AA820977	599	
A1455565	566	
dTPR2 (GH09432) GenBank submitt	1438	G C T A C A A T G A T C T G G A G A A G T T C G A G G A G T C G G T G G C G G A C T A T G A G A C G
		1487
AA950779	581	
A1456556	274	
A1457049	566	
A1110008	679	
AA201154	530	
AA952015	783	
A1516974	678	
AA201429	367	
AA392620	535	
AA439655	570	
A1544064	689	
A1516760	532	
AA978757	480	
AA202799	368	
AA735495	504	
AA201527	371	
AA438619	378	
A1516274	692	
AA440018	636	
AA820977	599	
A1455565	566	
dTPR2 (GH09432) GenBank submitt	1488	G C G C T G C A G C T G G A G A A G A C G C C G G A G A T T A A G C G A A T G C T G C G C G A G G C
		1537
AA950779	581	
A1456556	274	
A1457049	566	
A1110008	679	
AA201154	530	
AA952015	783	
A1516974	678	
AA201429	367	
AA392620	535	
AA439655	570	
A1544064	689	
A1516760	532	
AA978757	480	
AA202799	368	
AA735495	504	
AA201527	371	
AA438619	378	
A1516274	692	
AA440018	636	
AA820977	599	
A1455565	566	
dTPR2 (GH09432) GenBank submitt	1538	C A A G T T T G C G T T G A A G A A G T C G A A G C G A A A G G A C T A C T A C A A G A T C C T G G
		1587

FIGURE 11A (Continued)

AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	682	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1638	G C A T T G G A C G C A A T G C G T C C G A C G A G A T C A A G A A G G C G T A T C G C A A A
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	682	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1638	A A G G C G C T T G G T A C A T C A T C C G G A T C G A C A C G C A A A C A G C A G T G C C G A G G A
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	682	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1688	G C G C A A G G A G G A G G C T C A A G T T C A A G G A G G T G G G C G A G G C G T A C G C C A
AA950779	581	580
A1456556	274	273
A1457049	586	585
A1110008	679	678
AA201154	530	529
AA952015	783	782
A1516974	678	677
AA201429	387	386
AA392620	535	534
AA439655	570	569
A1544064	689	688
A1516760	532	531
AA978757	480	479
AA202799	388	387
AA735495	504	503
AA201527	371	370
AA438619	378	377
A1516274	682	691
AA440018	636	635
AA820977	589	588
A1455565	586	585
dTPR2 (GH09432) GenBank submit	1738	T A C T G T C G G A T G C T C A C A A G A A G T C G C G C T A C G A C A G C G G C C A G G A T A T C

FIGURE 11A (Continued)

AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	367	366
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	589	588
AI455565	566	565
dTPR2 (GH09432) GenBank submit	1789	G A G G A G C A G G A G C A A G G C G A C T T C G A T C C G A A T C A A A T G T T C C G C A C A T T 1837
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	367	366
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	589	588
AI455565	566	565
dTPR2 (GH09432) GenBank submit	1838	C T T C C A A T T C A A C G G G G G T G G C C G G A A T A A T T C A T C G T T C A A C T T T G A G T 1887
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	367	366
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	589	588
AI455565	566	565
dTPR2 (GH09432) GenBank submit	1888	T C T A G G A T C C C A A C G A G T G T T G T T C A C C A C C A C A G A G A A G A C C A T C T 1937
AA950779	581	580
AI456556	274	273
AI457049	566	565
AI110008	679	678
AA201154	530	529
AA952015	783	782
AI516974	678	677
AA201429	367	366
AA392620	535	534
AA439655	570	569
AI544064	689	688
AI516760	532	531
AA978757	480	479
AA202799	368	367
AA735495	504	503
AA201527	371	370
AA438619	378	377
AI516274	692	691
AA440018	636	635
AA820977	589	588
AI455565	566	565
dTPR2 (GH09432) GenBank submit	1938	C A A T C C C A T A C T T T C T G C C T C A T C C G A A A C C A A C A T A C A G C A G C G C A C A A 1987

FIGURE 11A (Continued)

AA950779	581	580	
AI456556	274	273	
AI457049	566	565	
AI110008	679	678	
AA201154	530	529	
AA952015	783	782	
AI516974	678	677	
AA201429	387	386	
AA392620	535	534	
AA439655	570	569	
AI544064	689	688	
AI516760	532	531	
AA978757	480	479	
AA202799	368	367	
AA735495	504	503	
AA201527	371	370	
AA438619	378	377	
AI516274	692	691	
AA440018	636	635	
AA820977	599	598	
AI455565	566	565	
dTPR2 (GH09432) GenBank submitt	1988	A T T T T G A A C T C T T T A C A T A T T C T T T C C A A A A A G C A A G A A A A T A C C A C	2037
AA950779	581	580	
AI456556	274	273	
AI457049	566	565	
AI110008	679	678	
AA201154	530	529	
AA952015	783	782	
AI516974	678	677	
AA201429	387	386	
AA392620	535	534	
AA439655	570	569	
AI544064	689	688	
AI516760	532	531	
AA978757	480	479	
AA202799	368	367	
AA735495	504	503	
AA201527	371	370	
AA438619	378	377	
AI516274	692	691	
AA440018	636	635	
AA820977	599	598	
AI455565	566	565	
dTPR2 (GH09432) GenBank submitt	2038	A T T T T G A T T A T G T T A C G A A T G A A T A T A T G C C A A G T T A T T G A A A A A A T A	2087
AA950779	581	580	
AI456556	274	273	
AI457049	566	565	
AI110008	679	678	
AA201154	530	529	
AA952015	783	782	
AI516974	678	677	
AA201429	387	386	
AA392620	535	534	
AA439655	570	569	
AI544064	689	688	
AI516760	532	531	
AA978757	480	479	
AA202799	368	367	
AA735495	504	503	
AA201527	371	370	
AA438619	378	377	
AI516274	692	691	
AA440018	636	635	
AA820977	599	598	
AI455565	566	565	
dTPR2 (GH09432) GenBank submitt	2088	T T C T A A A T C A A A A T A A T G C A A C T A A A T T T C C A G T G T A A G T T C A C A T T T T T	2137
AA950779	581	580	
AI456556	274	273	
AI457049	566	565	
AI110008	679	678	
AA201154	530	529	
AA952015	783	782	
AI516974	678	677	
AA201429	387	386	
AA392620	535	534	
AA439655	570	569	
AI544064	689	688	
AI516760	532	531	
AA978757	480	479	
AA202799	368	367	
AA735495	504	503	
AA201527	371	370	
AA438619	378	377	
AI516274	692	691	
AA440018	636	635	
AA820977	599	598	
AI455565	566	565	
dTPR2 (GH09432) GenBank submitt	2138	A A T G T T C T T T C T T G G A T T T T T T C G G C A A C A T T A A T A A T C A T G G G A	2187

FIGURE 11A (Continued)

FIGURE 11B

ClustalW Formatted Alignments

FIGURE 11B (Continued)

AI456052	90	CATGATGCTGCTGACCTATATAAGCGCCCTGACCGACGCCCGAACACGGCCAA	GACGCCCGAACACGGCCAA	A	12
AA391402	120	CATGATGCTGCTGACCTATATAAGCGCCCTGACCGACGCCCGAACACGGCCAA	GACGCCCGAACACGGCCAA	A	16
AI514849	201	CATGATGCTGCTGACCTATATAAGCGCCCTGACCGACGCCCGAACACGGCCAA	GACGCCCGAACACGGCCAA	A	20
AI519786	200	CATGATGCTGCTGACCTATATAAGCGCCCTGACCGACGCCCGAACACGGCCAA	GACGCCCGAACACGGCCAA	A	20
AI546378	97	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	140
AA438290	106	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA820520	106	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA941597	106	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AI455870	106	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA950277	106	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA941028	110	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA951077	115	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA951083	122	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA949900	122	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA541065	123	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AA441093	123	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
dTPR2 (GH09432) GenBank submitt	153	GTTAATCGCA-TCGAGGGCACCAGAAATCGGG-	-GATTCTAACACGGCGG	G	15
AI456052	140	TACCGCATCGATCCGGGCTTCGAGAAGGGCTACGTCGCTCGTGTGGCCAAAGGTGC	188		
AA391402	170	TACCGCATCGATCCGGGCTTCGAGAAGGGCTACGTCGCTCGTGTGGCCAAAGGTGC	218		
AI514849	251	TACCGCATCGATCCGGGCTTCGAGAAGGGCTACGTCGCTCGTGTGGCCAAAGGTGC	300		
AI519786	250	TACCGCATCGATCCGGGCTTCGAGAAGGGCTACGTCGCTCGTGTGGCCAAAGGTGC	298		
AI546378	144	CCCGTGGGACGCTACATACATACGGCACGGCACACACACACACACACACACATTAC	165		
AA438290	153	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACACATTAC	202		
AA820520	153	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACACATTAC	201		
AA941597	153	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	202		
AI455870	153	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	202		
AA950277	153	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	202		
AA941028	157	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	207		
AA951077	162	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	211		
AA951083	169	[REDACTED]	1/1		
AA949900	169	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACACATTAC	212		
AA541065	170	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACATTAC	212		
AA441093	170	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACATTAC	212		
dTPR2 (GH09432) GenBank submitt	200	GGGGTGCGACGCTACATACATACGGCACGGCACACACACACACACATTAC	212		
AI456052	190	TGTCTGGCCCCCTGGGGCGCATATTTTGGCACCCGAACAGGGCCGTCAAAAATGGT	23		
AA391402	220	TGTCTGGCCCCCTGGGGCGCATATTTTGGCACCCGAACAGGGCCGTCAAAAATGGT	26		
AI514849	301	TGTCTGGCCCCCTGGGGCGCATATTTTGGCACCCGAACAGGGCCGTCAAAAATGGT	35		
AI519786	300	TGTCTGGCCCCCTGGGGCGCATATTTTGGCACCCGAACAGGGCCGTCAAAAATGGT	34		
AI546378	194	TGGCCATTGCGAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	24		
AA438290	203	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AA820520	203	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AA941597	203	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AI455870	203	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AA950277	203	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AA941028	207	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	25		
AA951077	212	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	24		
AA951083	171	[REDACTED]	17		
AA949900	219	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	26		
AA541065	219	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	26		
AA441093	220	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	26		
dTPR2 (GH09432) GenBank submitt	250	TGGCCATTGCGCAAAAGCGCAAAAGCGAGTGGAAATAAAGGGGAAATTGAC	26		
AI456052	240	CAACCGAGCTGAATTCTGCTTTAGCAC-GGCTGTCTGGCTGCGCGAACAGACCGGG	285		
AA391402	270	CAACCGAGCTGAATTCTGCTTTAGCAC-GGCTGTCTGGCTGCGCGAACAGACCGGG	318		
AI514849	351	CAACCGAGCTGAATTCTGCTTTAGCAC-GGCTGTCTGGCTGCGCGAACAGACCGGG	344		
AI519786	350	CAACCGAGCTGAATTCTGCTTTAGCAC-GGCTGTCTGGCTGCGCGAACAGACCGGG	366		
AI546378	243	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	291		
AA438290	252	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	37		
AA820520	252	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	303		
AA941597	252	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	304		
AI455870	252	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	304		
AA950277	252	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	304		
AA941028	256	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGGCTGTCTTTC	34		
AA951077	247	[REDACTED]	24		
AA951083	171	[REDACTED]	17		
AA949900	268	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	311		
AA541065	268	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	311		
AA441093	269	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	317		
dTPR2 (GH09432) GenBank submitt	299	AAATAACAAACGTTTGTGCAAG-CACTGGACTCTGGCTGGCTGTCTTTC	347		

FIGURE 11B (Continued)

AI456052
 AA391402
 A1514849
 AI519786
 A1546378
 AA438290
 AA820520
 AA941597
 A1455870
 AA950277
 AA941028
 AA951077
 AA951083
 AA949900
 AA541065
 AA441093
 dTPR2 (GH09432) GenBank submitt

289 G C G C A A A T G T G C G C A A T T G G A G G C C A C C A T T C A G G C G A A C T A C G A T A C C 351
 310 G C G C A A A A G T G C C C C A A - T T G G A G G G C C A C C A T T C A G G C G A A C T A C G A T A C C 367
 400 G C G C A A A A G T T G C G C C A A T T G G A G G G C C A C C A T T C A G G C G A A C T A C G A T A C C 448
 399 G C G C A A A A G T T G C G C C A A T T G G A G G G C C A C C A T T C A G G C G A A C T A C G A T A C C 448
 292 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 341
 310 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 35
 301 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 35
 301 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 35
 301 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 35
 302 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 351
 305 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 351
 247
 171
 317 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 36
 317 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 36
 318 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 367
 348 A T T T T G T A A T T G C C A C C C A T T G G A G G G C C A G G T A A T T G A A A T T A G C G A C A C G 367

AI456052
 AA391402
 A1514849
 AI519786
 A1546378
 AA438290
 AA820520
 AA941597
 A1455870
 AA950277
 AA941028
 AA951077
 AA951083
 AA949900
 AA541065
 AA441093
 dTPR2 (GH09432) GenBank submitt

339 G A A A T T C C T A T C G C A T T G T G G G T C T C T A T T T G G A T A G T G C C T T G G A A A T T T G G 388
 368 G A A A T T C C T A T C G C A T T G T G G G T C T C T A T T T G G A T A G T G C C T T G G A A A T T T G G 417
 450 G A A A T T C C T A T C G C A T T G t G G G T C T C T A T T T G G A T A G T G C C T T G G A A A T T T G G 499
 449 G A A A T T C C T A T C G C A T T G t G G G T C T C T A T T T G G A T A G T G C C T T G G A A A T T T G G 496
 342 C G A A A T T C C T A T C G C A T T G G G A A G G A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A T T A A 388
 351 C A - - - - C G G G A C A G A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 395
 351 C A - - - - C G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 395
 351 C A T - - - - C G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 397
 351 C G A T - - - - C G G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 397
 352 C A G - - - - C G G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 397
 355 C A G - - - - C G G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 401
 247
 171
 367 A T A - - - - C G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 41
 367 C A - - - - C G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 41
 368 C A - - - - C G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 411
 368 C G A A - - - - C G G G G A A G A A A A C C T C A T C G A A C T C C G A A A T T G G A T G T G G G A A A T T A A 411

AI456052
 AA391402
 A1514849
 AI519786
 A1546378
 AA438290
 AA820520
 AA941597
 A1455870
 AA950277
 AA941028
 AA951077
 AA951083
 AA949900
 AA541065
 AA441093
 dTPR2 (GH09432) GenBank submitt

389 C G C C C C C T G T T T G A A T A T C G T T C T A C T C A A G G C T G A G T G C C T T G C A T T T 438
 418 C Q C C C C C T G T T T G A A A T A T C G T C T A C T C A A G G C T G A G T G C C T T G C A T T T 467
 500 C G C C C C C C T G T T T G A A A T A T C G T C T A C T C A A G G C T G A G T G C C T T G C A T T T 541
 499 C G C C C C C C T G T T T G A A A T A T C G T C T A C T C A A G G C T G A G T G C C T T G C A T T T 541
 389 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 438
 396 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 442
 397 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 442
 388 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 443
 399 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 444
 398 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 444
 401 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 447
 247
 171
 413 C G A C A G A A C C G C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 451
 411 C G A C A G A A A C A G C C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 457
 412 C G A C A G A A A C A G C C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 458
 445 C G A C A G A A A C A G C C - - A A C C A T C G A T G - - T C A A A G C A G A G G C A A A T T G T G C C C 451

AI456052
 AA391402
 A1514849
 AI519786
 A1546378
 AA438290
 AA820520
 AA941597
 A1455870
 AA950277
 AA941028
 AA951077
 AA951083
 AA949900
 AA541065
 AA441093
 dTPR2 (GH09432) GenBank submitt

439 T T G G G C C A T T T G T G G G C C T T G G A C A T T T G C G G T C A G T G T A A T G A A A C T 482
 468 T T G G G C C A T T T G T G A G G G C C T T G G A C A T T T G C G G T C A G T G T A A T G A A A C T 517
 550 T T G G G C C A T T T G T G G G C C T T G G A C A T T T G C G G T C A G T G T A A T G A A A C T 599
 549 T T G G G C C A T T T G
 436 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 484
 443 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 491
 444 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 492
 445 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 492
 445 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 492
 445 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 492
 448 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 497
 247
 171
 460 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 504
 468 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 506
 459 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 507
 492 A - A G G A C C G G G G A A C C A T T G C C G A G G G A A G A A G A A A C T G G G C C A A C G A C C 540

FIGURE 11B (Continued)

AI456052 489 G G A T A C C A C A T C G G C G G A T G C G A T T A C G T G A G G A G G G T C T G C T G C T A C T
 AA391402 518 G G A T A C C A C A T C G G C G G A T G C G A T T A C G T G A G G A G G G T C T G C T G C T A C T
 AI514849 600 G G A T A C C A C A T C G G C G G A T G C G A T T A C G T G A
 AI519786 562
 AI546378 485 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA438290 492 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA820520 493 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA941597 494 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AI455870 494 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA950277 494 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA941028 498
 AA951077 247
 AA951083 171
 AA949900 509 A A T
 AA541065 507 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 AA441093 508 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C
 dTPR2 (GH09432) GenBank submitt 511 A A T A C A A G G C C C A G A A C T A T C A G A A T G C A C T C A A G C T C T A C A C G G G A T G C C

 AI456052 539 A C A C G G A C A A C C T G G A C A A G G G A A T T C T T C A T T T C G A G G G G G C C C T G A C C
 AA391402 568 A C A C G G A C A A C C T G G A C A A G G G A A T T C T T C A T T T C G A G G G G G C C C T G A C C
 AI514849 622
 AI519786 562
 AI546378 535 A T A T T C G G C
 AA438290 542 A T A T T C G G C T G T G T C C C G G A C T
 AA820520 543 A T A T T C G G C T G T G T C C C G G A C T C G G C G G G C A T A C T A T G G C A A T C G G G C C G C T G
 AA941597 544 A T A T T C G G C T G T G T C C C G G A C T C G G C G G G C A T A C T A T G G C A A T
 AI455870 544 A T A T T C G G C
 AA950277 488
 AA941028 247
 AA951077 171
 AA951083 171
 AA949900 512
 AA541065 557 A T A T T C G G C T G T G T C C C G G A C T C G G C G G G C A T A C T A T G G C A A T C G G G C C G C T G
 AA441093 558 A T A T T C G G C T G T G T C C C G G A C T C G G C G G G C A T A C T A T G G C A A T
 dTPR2 (GH09432) GenBank submitt 561 A T A T T C G G C T G T G T C C C G G A C T C G G C G G G C A T A C T A T G G C A A T C G G G C C G C T G

 AI456052 589 C
 AA391402 618 C T C G A C C C G G A C C A C T A C C A G T C C A A G C A G A T G C G C
 AI514849 632
 AI519786 562
 AI546378 542
 AA438290 561
 AA820520 593 C T A C A T G A T G C T G C T C A A C T A T A A T
 AA941597 583
 AI455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 607 C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C A C G
 AA441093 597
 dTPR2 (GH09432) GenBank submitt 641 C T A C A T G A T G C T G C T C A A C T A T A A T A G C G C C C T G A C C G A C G C C C G A C A C G

 AI456052 590
 AA391402 654
 AI514849 632
 AI519786 562
 AI546378 542
 AA438290 561
 AA820520 618
 AA941597 593
 AI455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 657 C C A T A C G C A T C G A T C C C G G G C T T C G A G A A G G G C T A C G T C C G T G T
 AA441093 597
 dTPR2 (GH09432) GenBank submitt 691 C C A T A C G C A T C G A T C C C G G G C T T C G A G A A G G G C T A C G T C C G T G T G G G C C A A G

FIGURE 11B (Continued)

AI456052	580	580
AA391402	654	654
AI514849	632	632
AI519786	562	562
AI546378	542	561
AA438290	561	541
AA820520	618	590
AA941597	583	617
AI455870	551	582
AA950277	547	560
AA941028	488	546
AA951077	247	487
AA951083	171	249
AA949900	512	171
AA541065	700	511
AA441093	597	684
dTPR2 (GH09432) GenBank submitt	741	T G C T G T C T G G C C C T G G G C G A C A T T A T T G G C A C C G A A C A G G C C G T C A A A A T
AI456052	580	580
AA391402	654	654
AI514849	632	632
AI519786	562	562
AI546378	542	541
AA438290	561	560
AA820520	618	617
AA941597	583	582
AI455870	551	551
AA950277	547	546
AA941028	488	487
AA951077	247	249
AA951083	171	171
AA949900	512	511
AA541065	700	684
AA441093	597	597
dTPR2 (GH09432) GenBank submitt	791	G G T C A A C G A G C T G A A T T C G C T T A G C A C G G C T G T T G C T G C C G A A C A G A C G G
AI456052	580	580
AA391402	654	654
AI514849	632	632
AI519786	562	562
AI546378	542	541
AA438290	561	560
AA820520	618	617
AA941597	583	582
AI455870	551	551
AA950277	547	546
AA941028	488	487
AA951077	247	249
AA951083	171	171
AA949900	512	511
AA541065	700	684
AA441093	597	597
dTPR2 (GH09432) GenBank submitt	841	C G G C G C A A A A G T T G C G C C A A T T G G A G G C C A C C A T T C A G G C G A A C T A C G A T
AI456052	580	580
AA391402	654	654
AI514849	632	632
AI519786	562	562
AI546378	542	541
AA438290	561	560
AA820520	618	617
AA941597	583	582
AI455870	551	551
AA950277	547	546
AA941028	488	487
AA951077	247	249
AA951083	171	171
AA949900	512	511
AA541065	700	684
AA441093	597	597
dTPR2 (GH09432) GenBank submitt	891	A C G A A A T C C T A T C G C A A T G T G G T C T T C T A T T T G G A T A G T G C C T T G A A A T T

FIGURE 11B (Continued)

A1456052 590
 AA391402 654
 A1514849 632
 A1519786 562
 A1546378 542
 AA438290 561
 AA820520 618
 AA941597 583
 A1455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 700
 AA441093 597
dTPR2 (GH09432) GenBank submitt 941 G G C G C C C G C C T G T T T G A A A T A T C G T C T A C T C A A G G C T G A G T G C C T T G C A T 989

 A1456052 590
 AA391402 654
 A1514849 632
 A1519786 562
 A1546378 542
 AA438290 561
 AA820520 618
 AA941597 583
 A1455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 700
 AA441093 597
dTPR2 (GH09432) GenBank submitt 991 T T T T G G G G C G A T G T G A T G A G G C C T T G G A C A T T G C G G T C A G T G T A A T G A A A 1039

 A1456052 590
 AA391402 654
 A1514849 632
 A1519786 562
 A1546378 542
 AA438290 561
 AA820520 618
 AA941597 583
 A1455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 700
 AA441093 597
dTPR2 (GH09432) GenBank submitt 1041 C T G G A T A C C A C A T C G G G C G G A T G C G A T A T A C G T G A G A G G T C T G T G C C T G T A 1089

 A1456052 590
 AA391402 654
 A1514849 632
 A1519786 562
 A1546378 542
 AA438290 561
 AA820520 618
 AA941597 583
 A1455870 551
 AA950277 547
 AA941028 488
 AA951077 247
 AA951083 171
 AA949900 512
 AA541065 700
 AA441093 597
dTPR2 (GH09432) GenBank submitt 1091 C T A C A C G G A C A A C C T G G A C A A G G G A A T T C T T C A T T T C G A G C G G C C C T G A 1149

FIGURE 11B (Continued)

AI456052	590	52
AA391402	654	57
AI514849	632	58
AI519786	562	59
AI546378	542	60
AA438290	561	61
AA820520	618	62
AA941597	583	63
AI455870	551	64
AA950277	547	65
AA941028	488	66
AA951077	247	67
AA951083	171	68
AA949900	512	69
AA541065	700	70
AA441093	597	71
dTPR2 (GH09432) GenBank submitt	1141	C C C T C G A C C C G G A C C A C T A C A A G T C C A A G C A G A T G C G C A G C A A A T G C A A G
AI456052	590	52
AA391402	654	55
AI514849	632	56
AI519786	562	57
AI546378	542	58
AA438290	561	59
AA820520	618	60
AA941597	583	61
AI455870	551	62
AA950277	547	63
AA941028	488	64
AA951077	247	65
AA951083	171	66
AA949900	512	67
AA541065	700	68
AA441093	597	69
dTPR2 (GH09432) GenBank submitt	1191	C A G C T C A A G G A G A T G A A G G A G A A C G G C A A T A T G C T A T T C A A G T C G G G T C G
AI456052	590	52
AA391402	654	55
AI514849	632	56
AI519786	562	57
AI546378	542	58
AA438290	561	59
AA820520	618	60
AA941597	583	61
AI455870	551	62
AA950277	547	63
AA941028	488	64
AA951077	247	65
AA951083	171	66
AA949900	512	67
AA541065	700	68
AA441093	597	69
dTPR2 (GH09432) GenBank submitt	1241	G T A T C G C G A G G G C A C A C G T T A T C T A C A C G G A C G C C C T G A A G A T C G A T G A A C
AI456052	590	52
AA391402	654	55
AI514849	632	56
AI519786	562	57
AI546378	542	58
AA438290	561	59
AA820520	618	60
AA941597	583	61
AI455870	551	62
AA950277	547	63
AA941028	488	64
AA951077	247	65
AA951083	171	66
AA949900	512	67
AA541065	700	68
AA441093	597	69
dTPR2 (GH09432) GenBank submitt	1291	A C A A C A A G G A T A T C A A T T C G A A A T T G C T T T A C A A T C G G G C T T T G G T C A A C

FIGURE 11B (Continued)

AI456052	590	
AA391402	654	
AI514849	632	
AI519786	562	
AI546378	542	
AA438290	561	
AA820520	618	
AA941597	583	
AI455870	551	
AA950277	547	
AA941028	488	
AA951077	247	
AA951083	171	
AA949900	512	
AA541065	700	
AA441093	597	
dTPR2 (GH09432) GenBank submitt	1341	A C G C G T A T T G G C A A T T T G C G A G A G G C C G T G G C C G A T T G C A A T C G A G T G C T
AI456052	590	
AA391402	654	
AI514849	632	
AI519786	562	
AI546378	542	
AA438290	561	
AA820520	618	
AA941597	583	
AI455870	551	
AA950277	547	
AA941028	488	
AA951077	247	
AA951083	171	
AA949900	512	
AA541065	700	
AA441093	597	
dTPR2 (GH09432) GenBank submitt	1391	G G A G C T G A A T A G T C A G T A T C T G A A G G C T C T G T T G C T G C G A G C G C G C G C T G C T
AI456052	590	
AA391402	654	
AI514849	632	
AI519786	562	
AI546378	542	
AA438290	561	
AA820520	618	
AA941597	583	
AI455870	551	
AA950277	547	
AA941028	488	
AA951077	247	
AA951083	171	
AA949900	512	
AA541065	700	
AA441093	597	
dTPR2 (GH09432) GenBank submitt	1441	A C A A T G A T C T G G A G A G T T C G A G G A G T C G G T G G C G G A C T A T G A G A C G G C G
AI456052	590	
AA391402	654	
AI514849	632	
AI519786	562	
AI546378	542	
AA438290	561	
AA820520	618	
AA941597	583	
AI455870	551	
AA950277	547	
AA941028	488	
AA951077	247	
AA951083	171	
AA949900	512	
AA541065	700	
AA441093	597	
dTPR2 (GH09432) GenBank submitt	1491	C T G C A G C T G G A G A G A C G C C G G A G A T T A A G C G A A T G C T G C G C G A G G C C A A

FIGURE 11B (Continued)

A1456052	590	52
AA391402	654	65
A1514849	632	61
A1519786	562	46
A1546378	542	26
AA438290	561	24
AA820520	618	17
AA941597	583	32
A1455870	551	57
AA950277	547	52
AA941028	488	2
AA951077	247	24
AA951083	171	24
AA949900	512	5
AA541065	700	61
AA441093	597	54
dTPR2 (GH09432) GenBank submitt	1541	G T T T G C G T T G A A G A A G T C G A A G C G A A A G G A C T A C T A C A A G A T C C T G G G C A
A1456052	590	581
AA391402	654	651
A1514849	632	631
A1519786	562	561
A1546378	542	5
AA438290	561	99
AA820520	618	617
AA941597	583	2
A1455870	551	62
AA950277	547	53
AA941028	488	4
AA951077	247	24
AA951083	171	17
AA949900	512	511
AA541065	700	61
AA441093	597	56
dTPR2 (GH09432) GenBank submitt	1591	T T G G A C G C A A T G C G T C C G A C G A C G A G A T C A A G A A G G C G T A T C G C A A A A A G
A1456052	590	581
AA391402	654	651
A1514849	632	631
A1519786	562	561
A1546378	542	5
AA438290	561	99
AA820520	618	617
AA941597	583	2
A1455870	551	62
AA950277	547	53
AA941028	488	4
AA951077	247	24
AA951083	171	17
AA949900	512	511
AA541065	700	61
AA441093	597	56
dTPR2 (GH09432) GenBank submitt	1641	G C G C T G G T A C A T C A T C C G G A T C G A C A C G C A A A C A G C A G T G C C G A G G A G C G
A1456052	590	581
AA391402	654	651
A1514849	632	631
A1519786	562	561
A1546378	542	5
AA438290	561	99
AA820520	618	61
AA941597	583	2
A1455870	551	62
AA950277	547	53
AA941028	488	4
AA951077	247	24
AA951083	171	17
AA949900	512	511
AA541065	700	61
AA441093	597	56
dTPR2 (GH09432) GenBank submitt	1691	C A A G G A G G A G G A G C T C A A G T T C A A G G A G G T G G G C G A G G C G T A C G C C A T A C

FIGURE 11B (Continued)

<i>AI456052</i>	590	58
<i>AA391402</i>	654	61
<i>AI514849</i>	632	57
<i>AI519786</i>	562	51
<i>AI546378</i>	542	48
<i>AA438290</i>	561	44
<i>AA820520</i>	618	40
<i>AA941597</i>	583	36
<i>AI455870</i>	551	32
<i>AA950277</i>	547	28
<i>AA941028</i>	488	24
<i>AA951077</i>	247	20
<i>AA951083</i>	171	16
<i>AA949900</i>	512	12
<i>AA541065</i>	700	8
<i>AA441093</i>	597	4
<i>dTPR2 (GH09432) GenBank submitt</i>	1741	T G T C G G A T G C T C A C A A G A A G T C G C G C T A C G A C A G C G G C C A G G A T A T C G A G
<i>AI456052</i>	590	58
<i>AA391402</i>	654	61
<i>AI514849</i>	632	57
<i>AI519786</i>	562	51
<i>AI546378</i>	542	48
<i>AA438290</i>	561	44
<i>AA820520</i>	618	40
<i>AA941597</i>	583	36
<i>AI455870</i>	551	32
<i>AA950277</i>	547	28
<i>AA941028</i>	488	24
<i>AA951077</i>	247	20
<i>AA951083</i>	171	16
<i>AA949900</i>	512	12
<i>AA541065</i>	700	8
<i>AA441093</i>	597	4
<i>dTPR2 (GH09432) GenBank submitt</i>	1791	G A G C A G G A G C A A G C C G A C T T C G A T C C G A A T C A A A T G T T C C G C A C A T T C T T
<i>AI456052</i>	590	58
<i>AA391402</i>	654	61
<i>AI514849</i>	632	57
<i>AI519786</i>	562	51
<i>AI546378</i>	542	48
<i>AA438290</i>	561	44
<i>AA820520</i>	618	40
<i>AA941597</i>	583	36
<i>AI455870</i>	551	32
<i>AA950277</i>	547	28
<i>AA941028</i>	488	24
<i>AA951077</i>	247	20
<i>AA951083</i>	171	16
<i>AA949900</i>	512	12
<i>AA541065</i>	700	8
<i>AA441093</i>	597	4
<i>dTPR2 (GH09432) GenBank submitt</i>	1841	C C A A T T C A A C G G C G G T G G C C G G A A T A A T T C A T C G T T C A A C T T T G A G T T C T
<i>AI456052</i>	590	58
<i>AA391402</i>	654	61
<i>AI514849</i>	632	57
<i>AI519786</i>	562	51
<i>AI546378</i>	542	48
<i>AA438290</i>	561	44
<i>AA820520</i>	618	40
<i>AA941597</i>	583	36
<i>AI455870</i>	551	32
<i>AA950277</i>	547	28
<i>AA941028</i>	488	24
<i>AA951077</i>	247	20
<i>AA951083</i>	171	16
<i>AA949900</i>	512	12
<i>AA541065</i>	700	8
<i>AA441093</i>	597	4
<i>dTPR2 (GH09432) GenBank submitt</i>	1891	A G G A T C C C A A C G A G T G T T G T T C A C C A C C A C A G A G A A G A A G A C C A T C T C A A

FIGURE 11B (Continued)

AI456052	590	585
AA391402	654	657
AI514849	632	637
AI519786	562	567
AI546378	542	547
AA438290	561	564
AA820520	618	620
AA941597	583	587
AI455870	551	552
AA950277	547	548
AA941028	488	489
AA951077	247	248
AA951083	171	172
AA949900	512	513
AA541065	700	701
AA441093	597	598
dTPR2 (GH09432) GenBank submitt	1941	T C C C A T A C T T T C T G C C T C A T C C G A A A C C A A C A T A C A G C A G C G C A C A A A T T
AI456052	590	585
AA391402	654	657
AI514849	632	637
AI519786	562	567
AI546378	542	547
AA438290	561	564
AA820520	618	620
AA941597	583	587
AI455870	551	552
AA941028	488	489
AA951077	247	248
AA951083	171	172
AA949900	512	513
AA541065	700	701
AA441093	597	598
dTPR2 (GH09432) GenBank submitt	1991	T T G A A C T C T T T A C A T A T T T C T T T C C A A A A A G C A A G A A A A T A C C A C A T T
AI456052	590	585
AA391402	654	657
AI514849	632	637
AI519786	562	567
AI546378	542	547
AA438290	561	564
AA820520	618	620
AA941597	583	587
AI455870	551	552
AA950277	547	548
AA941028	488	489
AA951077	247	248
AA951083	171	172
AA949900	512	513
AA541065	700	701
AA441093	597	598
dTPR2 (GH09432) GenBank submitt	2041	T T G A T T A T G T T A A C G A A T G A A T A T A T G C C A A G T T A T T T G A A A A A A T A T T C
AI456052	590	585
AA391402	654	657
AI514849	632	637
AI519786	562	567
AI546378	542	547
AA438290	561	564
AA820520	618	620
AA941597	583	587
AI455870	551	552
AA950277	547	548
AA941028	488	489
AA951077	247	248
AA951083	171	172
AA949900	512	513
AA541065	700	701
AA441093	597	598
dTPR2 (GH09432) GenBank submitt	2091	T A A A T C A A A A T A A T G C A A C T A A A T T T C C A G T G T A A G T T C A C A T T T T A A A

FIGURE 11B (Continued)

AI456052	580	580	
AA391402	654	653	
AI514849	632	631	
AI519786	562	561	
AI546378	542	541	
AA438290	561	550	
AA820520	618	549	
AA941597	583	548	
AI455870	551	547	
AA950277	547	546	
AA941028	488	487	
AA951077	247	246	
AA951083	171	170	
AA949900	512	511	
AA541065	700	699	
AA441093	567	566	
dTPR2 (GH09432) GenBank submitt	2141	T G T T C T T T C T T G G A T T T T T T T C G G C A A C A T T A A T A A T C A T G G G A G A T	2140

FIGURE 12

ClustalW Formatted Alignments

AA950937 1
 AI113626 1
 AA978719 1
 AI260802 1
 AI541593 1
 AI541599 1
 AA695052 1
 AI107772 1
 AI113621 1
 AI515548 1
 AI532198 1
 AI389024 1
 AA391063 1
 AA940727 1
 AA802232 1
 AI531980 1
 dmfl cDNA only 1
 AA264488 1

AT T A T T C G T G A A A A T T T C T G C A T A C G G A A A G A G G A A A 28
 A A A G A A A A T T T C G T G A A A A T T T C T G C A T A C G G A A A G A G G A A A 35
 A A A G A A A A T T T C G T G A A A A T T T C T G C A T A C G G A A A G A G G A A A 42
 A C A A A G A A A A A T T C G T G A A A A A T T C T G C A T A C G G A A A G A G G A A A 44
 T G C A C A A G A A A A A T T C G T G A A A A A T T C T G C A T A C G G A A A G A G G A A A 45
 T G A C A A A G A A A A A T T C G T G A A A A A T T C T G C A T A C G G A A A G A G G A A A 46
 G G C A C G A G G G A A A A A T T C G T G A A A A A T T C T G C A T A C G G A A A G A G G A A A 47
 G G C A C G A G G A A A A A T T C G T G A A A A A T T C T G C A T A C G G A A A G A G G A A A 48

AGA A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 21
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 29
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 37
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 45
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 53
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 61
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 69
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 77
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 85
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 93
 AT T C G A G C A A C A G A A A G C C A A C A C A A T C C A C A A A A A T G T C T T T A T T C G G A 100

GC G T T G A T G G G T G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 71
 G C G T T G A T G G G T G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 79
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 87
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 95
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 103
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 111
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 119
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 127
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 135
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 143
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 151
 G C G T T G A T G G G C G A T T T C G A C G A C G A T C T C G G C T T A T G A A C A A C C A C A T 159

G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 121
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 129
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 137
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 145
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 153
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 160
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 168
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 176
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 184
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 192
 G A A C C A C A C T A T G A A C G C G A T G A A C A T G C A G A T G C G C T C G A T G A A T C G G C 200

FIGURE 12 (Continued)

AA950937	122	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCCCTTGAC	171
AI113626	140	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	180
AA978719	154	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	193
AI260802	159	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	208
AI541593	161	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	210
AI541599	161	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	210
AA695052	161	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	210
AI107772	161	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	210
AI113621	161	TGATGARCAAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	210
AI515548	180	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	229
AI532198	186	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	235
AI389024	189	TGATGARCAAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	238
AA391063	193	TGATGARCAAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	242
AA940727	195	TGATGARCAAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	244
AA802232	196	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	245
AI531980	197	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	247
dmfl cDNA only	198	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	250
AA264488	201	TGATGAACAGCTTATGCCCGATCCCTCATGGAGGCTCTTGAC	

AA950937	172	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	220
A I 113626	180	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	238
A A 978719	204	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	252
A I 260802	209	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 541593	211	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 541599	211	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A A 695052	211	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 107772	211	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 113621	211	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 515548	230	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	259
A I 532198	236	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	278
A I 389024	239	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	284
A A 391063	243	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	287
A A 940727	245	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	293
A A 802232	246	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	294
A I 531980	247	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	294
dmlf cDNA only	248	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	295
AA264488	251	C A G G G A T T C C A G C A -	G A A C G C T C T C A T G G A G C C G T C C C C A G A T G C C G G C C A	295

AA950937	221	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A C A A A C T T T A A 266
AI113626	239	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 267
AA978719	253	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 268
AI260802	258	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 269
AI541593	260	T G C C A G C C A T G G G A A C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 303
AI541599	260	T G C C A G C C A T G G G A A C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 305
AA695052	260	T G C C A G C C A T G G G A A C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 305
AI107772	260	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 305
AI113621	260	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 305
AI515548	279	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 324
A1532198	285	T G C C A G C C A T G G G A A C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 333
A1389024	288	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 333
AA391063	293	T G C - A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 338
AA940727	294	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 338
AA802232	295	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 342
A1531980	296	T G C C A G C C A T G G G A A C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 344
dmfl cDNA only	298	T G C C A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 347
AA264488	299	T G C - A G C C A T G G G C T C T C G G C A T G C C C A - - - T G A T G C C A - - A A C T T T A A 348

AA950937	269	T C G G C C T C G I T G A A C G G C T G A T A I T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	31
A1113626	285	T C G G C C T G T T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	33
AA978719	299	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	34
A1260802	304	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	34
A1541593	305	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	35
A1541599	306	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	35
AA6950502	306	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	35
A1107772	306	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	35
A1113621	306	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	35
A1515548	325	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	37
A1532198	331	T C G G C C T G [A] T G A A C G C T G C [A] T T T G G T G G [G] A T T C A G G G C G C A T C C T T C T G C C	38
A1389024	334	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	38
AA391063	336	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	38
AA940727	340	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	38
AA802232	341	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	39
A1531980	342	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	39
dmlf cDNA only	343	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	39
AA264488	344	T C G G C C T G T G A A C G C T G A T A T T G G T G G C A A T T C A G G G C G C A T C C T T C T G C C	39

FIGURE 12 (Continued)

AA950937	319	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	367
AI113626	335	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	383
AA978719	349	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	397
AI260802	354	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	402
AI541593	355	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	404
AI541599	356	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	404
AA695052	356	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	404
AI107772	356	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	404
AI113621	356	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	404
AI515548	375	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	423
AI532198	381	AGAGCACCCTGGGATGACCATGTCATCGGGTTCCTTGCGATGGGCG-	TGCTCAGAT	426
AI389024	384	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	432
AA391063	386	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCGGTTGCTCAGAT	TCCCTCAGAT	435
AA940727	389	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	438
AA802232	391	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	439
AI531980	392	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	440
dmif cDNA only	393	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCG-	TCCCTCAGAT	441
AA264488	394	AGAGCACCCTGATGACCATGTCATCGGGTCCCGATGGGCGTTGCTCAGAT	TGCTCAGAT	443
AA950937	368	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	417	
AI113626	384	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	433	
AA978719	388	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	447	
AI260802	403	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	452	
AI541593	405	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	454	
AI541599	405	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	454	
AA695052	405	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	454	
AI107772	405	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	454	
AI113621	405	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	454	
AI515548	424	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	473	
AI532198	430	CTACCAAGGCCAGCAGCACTAGTTGCGAAGGACCGGGAGGCATTTCGTGAGA	479	
AI389024	433	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	482	
AA391063	436	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	485	
AA940727	438	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	488	
AA802232	440	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	490	
AI531980	441	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	491	
dmif cDNA only	442	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	493	
AA264488	444	CTACCAAGGCCAGCAGCACTAGTACCAAACAGGACCGGGAGGCATTTCGTGAGA	493	
AA950937	418	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	467	
AI113626	434	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	483	
AA978719	448	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	497	
AI260802	453	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	502	
AI541593	455	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	504	
AI541599	455	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	504	
AA695052	455	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	504	
AI107772	455	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	504	
AI113621	455	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	504	
AI515548	474	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	523	
AI532198	490	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	497	
AI389024	483	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCCATT	532	
AA391063	486	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	535	
AA940727	489	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	538	
AA802232	490	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	539	
AI531980	491	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	540	
dmif cDNA only	492	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	541	
AA264488	494	CCCGCAGGACGGTGCAGGACTCGCCGCACTGGGTGAAGAACATGGCATT	543	
AA950937	488	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	517	
AI113626	494	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	533	
AA978719	498	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	547	
AI260802	503	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	552	
AI541593	505	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	554	
AI541599	505	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	554	
AA695052	505	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	554	
AI107772	505	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	554	
AI113621	505	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	554	
AI515548	524	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	573	
AI532198	498	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	497	
AI389024	533	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	582	
AA391063	536	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	540	
AA940727	539	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	588	
AA802232	540	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	589	
AI531980	541	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	591	
dmif cDNA only	542	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	592	
AA264488	544	GGTCATCACATCGGGGAGCGGGCAGCACATTATTGAGAAAAAGAGCAGGGACAT	592	

FIGURE 12 (Continued)

AA950937	518	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	567	
AI113626	534	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	563	
AA978719	548	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	567	
AI260802	553	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	602	
AI541593	555	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	599	
AI541599	555	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	604	
AA695052	555	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	585	
AI107772	555	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	591	
AI113621	555	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	604	
AI515548	574	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	623	
AI532198	498					497	
AI389024	583	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	632	
AA391063	541					540	
AA940727	589	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	638	
AA802232	590	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	639	
AI531980	591	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	627	
dmrl cDNA only	592	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	641	
AA264488	594	GGCGCTCAGGACAACTGGAGGA	GC	GCCAGGAGTTCATTAAATCTGGAGGA	GG	643	
AA950937	568	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	617
AI113626	564	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	563
AA978719	568	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	647
AI260802	603	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	652
AI541593	600						599
AI541599	595						585
AA695052	596						591
AI107772	592						608
AI113621	605	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	654
AI515548	624	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	673
AI532198	498						497
AI389024	633	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGGAG	682
AA391063	541						540
AA940727	639	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGTAG	661
AA802232	640	AAGAGCGACAGTTTGACAG	GG	GA	GT	TACATCGCG	675
AI531980	628						627
dmrl cDNA only	642	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGTAG	691
AA264488	644	GAGAAGCCAGCAGTTTGACAG	GG	GA	GT	TACATCGCGTAG	686
AA950937	618	G-CG TGCAGTCAAGACATCAT	G	C	TGGTGGCATGCA	GGCCATCATGCCCGC	666
AI113626	564						563
AA978719	648	GGCGTGCAGTCAAGACATCAT	G	C	TGGTGGCATGCA	GGCCATCATGCCCGC	697
AI260802	653	GGCGTGCAGTCAAGACATCAT	G	C	TGGTGGCATGCA	GGCCATCATGCCCGC	701
AI541593	600						599
AI541599	609						608
AA695052	596						585
AI107772	592						591
AI113621	655	GCG					657
AI515548	674	GCGGTGCAGTCAAGACATCAT	G	C	TGGTGGCATGCA	-GCCATCATG	717
AI532198	498						497
AI389024	683	GCG					685
AA391063	541						540
AA940727	682						681
AA802232	676						675
AI531980	628						627
dmrl cDNA only	692	GGCGTGCAGTCAAGACATCAT	G	C	TGGTGGCATGCA	GGCCATCATGCCCGC	741
AA264488	687						686
AA950937	667	C					667
AI113626	564						563
AA978719	688	CCGTCAGCGGGCACACAC	C	C	CGGATTCGACGTTGACCA	TGTGGAGGACG	747
AI260802	702	CCGTCAGCGGGCACACAC	C	C	CGGATTCGACGTTGACCA	TGTGGAGGACG	708
AI541593	600						599
AI541599	609						608
AA695052	596						585
AI107772	592						591
AI113621	659						657
AI515548	718						717
AI532198	498						497
AI389024	686						685
AA391063	541						540
AA940727	682						681
AA802232	676						675
AI531980	628						627
dmrl cDNA only	742	CCGTCAGCGGGCACACAC	C	C	CGGATTCGACGTTGACCA	TGTGGAGGACG	791
AA264488	687	CCGTCAGCGGGCACACAC	C	C	CGGATTCGACGTTGACCA	TGTGGAGGACG	686

FIGURE 12 (Continued)

AA950937	668		667
AI113626	564		563
AA978719	748	ACGACGACGATGATGC	763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmif cDNA only	792	ACGACGACGATGATGACTGTGTAATCCAGGAGCAGCAACC GGTT CGC	841
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmif cDNA only	842	TCCCTCCGGGGCCATTATTCCAGTGCGCCAACGGCACCGCAGAACAG	881
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmif cDNA only	892	ATATAATTACTAAATCTAAAGTCATAACAGTATATTTACTAACTATCCG	941
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmif cDNA only	942	ATAAAAACAGAACAGAATTGCACTATAAAATTCTGCTAATTACATTCC	991
AA264488	687		686

FIGURE 12 (Continued)

AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	586		585
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	992	CAACCTGCGTTCAAAACGAAACGAAATATCGAATCGAAATCATAGAACATGCACAA	1041
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	586		585
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1042	GAGCAGCATAACATCCACATCCCCATGCCGCCAATCCGAGGGCGCCAACAAAC	1081
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	586		585
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1092	GTGCCGTAAAAACATTTCACACGGAGGACGAAGCGGGCAGCTCCATACAAAG	1141
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	586		585
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	686		685
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1142	GCGGTCAGCGCGGGCAAGAAGAAGTAGTAGAACGTGATCATCTGTATGC	1191
AA264488	687		686

FIGURE 12 (Continued)

AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1192	1241
AA264488	687	686
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1242	1291
AA264488	687	686
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1292	1341
AA264488	687	686
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1342	1391
AA264488	687	686

FIGURE 12 (Continued)

AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	696		695
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1392	A A C A A C T T G C T T A A T T A C T T A A A A C A A A T G T G A C T A T T C A A C G C C G A A	1441
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	696		695
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1442	A T C A T T A C A A C A C A C A C T C T C A G A C C T A A T C G A A A A A T T C A A T G A A A G T A	1481
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	696		695
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1492	A T G G A A T A T A T A T G A A A T C G T A A T T A T A A G T T T G A A T T A T T G A T T A A T T	1541
AA264488	687		686
AA950937	668		667
AI113626	564		563
AA978719	764		763
AI260802	709		708
AI541593	600		599
AI541599	609		608
AA695052	596		595
AI107772	592		591
AI113621	658		657
AI515548	718		717
AI532198	498		497
AI389024	696		695
AA391063	541		540
AA940727	682		681
AA802232	676		675
AI531980	628		627
dmrl cDNA only	1542	C T C A A G T T T T A G A T T T G T T A G C C A C T A A G C T T T A A A T T A T G G A T G C C A	1591
AA264488	687		686

FIGURE 12 (Continued)

AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1592	1591
AA264488	697	696
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1642	1641
AA264488	697	696
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1692	1741
AA264488	697	696
AA950937	668	667
AI113626	564	563
AA978719	764	763
AI260802	709	708
AI541593	600	599
AI541599	609	608
AA695052	596	595
AI107772	592	591
AI113621	658	657
AI515548	718	717
AI532198	498	497
AI389024	696	695
AA391063	541	540
AA940727	682	681
AA802232	676	675
AI531980	628	627
dmrl cDNA only	1742	1753
AA264488	697	696

FIGURE 13A

hTPR2 Protein 484 amino acids

MAATEPELDDQEAKREAETFKEQGNAYYAKKDYN EA NY YT KAI DMCPKNA
SYYGNRAATLMMMLGRFREALGDAQQSVRLDDSFVRGHLREGKCHLSLGNAME
ACRSFQR ALELDHKNAQAQQEFKNANA VMEYEKIAETDFEKDRFRKVVF CMDR
ALEFAPACHRFKILKAEC LAM LG RY PEA QSVASDILRMDSTNADAL YVRGLCLY
YEDCIEKA VQFFVQALRMAPDHEKACIACRNAKALKAKKEDGNKAFKEGNYKL
AYELYTEALGIDPNNIKTNAKLYCNRGTVNSKLKLD DAI EDCTNAVKLDDTYI
KAYLRR AQCYMDTEQYEEAVRDYEK VYQTEKTKEH KQLLKNAQLELKKS KRK
DYYKILGVVDKNASEDEIKKAYRKRALMHH PDRHSGASAEVQKEEEKKFKEVGE
AFTILSDPKKKTRYDSQQLDEEGMNM GDFDPNNIFKAFFGGPGGF SFEASGP GNG
FFFQFG

FIGURE 13B

hTPR2 cDNA 1756 base pairs

CGGCTGCCGCGGAGTGCATGTGTAATGGCGCGACCGAGCCGGAGCTGCT
CGACGACCAAGAGGCAGAGAGGGAAAGCAGAGACTTCAAGGAACAAGGAAA
TGCATACTATGCCAAGAAAGATTACAATGAAGCTTATAATTATTATACAAAAA
GCCATAGATATGTGTCCTAAAAATGCTAGCTATTATGGTAATCGAGCAGCCA
CCTTGATGATGCTGGAAAGGTTCCGGGAAGCTCTGGAGATGCACAACAGTC
AGTGAGGTTGGATGACAGTTGTCCGGGGACATCTACGAGAGGGCAAGTGC
CACCTCTCTGGGGATGCCATGGCAGCATGTCG CAGCTTCCAGAGAGCCC
TAGAACTGGATCATAAAAATGCTCAGGCACAACAAGAGTTCAAGAATGCTAA
TGCAGTCATGGAATATGAGAAAATAGCAGAAACAGATTITGAGAACCGAGA
TTTCGGAAGGTTGTTCTGCATGGACC GTGCCCTAGAATTGCCCTGCCT
GCCATCGCTTCAAAATCTCAAGGCAGAAATGTTAGCAATGCTGGGTCGTTAT
CCGGAAAGCACAGTCTGGCTAGTGACATTCTACGAATGGATTCCACCAATG
CAGATGCTCTGTATGTACGGAGGTCTTGCCTTATTACGAAGATTGTATTGAG
AAGGCAGTT CAGTTT CGTACAGGCTCTCAGGATGGCCTGACCACGAGA
AGGCCTGCATTGCC TG CAGAAATGCCAAGCACTCAAAGCAAAGAAAG
ATGGGAATAAAGCATTAAAGGAAGGAAATTACAAACTAGCATATGAACTGTA
CACAGAAGCCCTGGGGATAGACCCAAACAATATAAAACAAATGCTAAACTC
TACTGTAATCGGGGTACGGTTAATTCCAAGCTTAGAAGACTAGATGATGCAA
TAGAAGACTGCACAAATGCAGTGAAGCTTGATGACACTTACATAAAAGCCTA
CTTGAGAAGAGCTCAGTGTACATGGACACAGAACAGTATGAAGAACAG
CGAGACTATGAAAAAGTATACAGACAGAGAAAACAAAGAACACAAACAG
CTCCTAAAAAATGCGCAGCTGGAACTGAAGAAGAGTAAGAGGAAAGATTAC
TACAAGATTCTAGGAGTGGACAAGAATGCCTCTGAGGACGAGATCAAGAAA
GCTTATCGGAAACGGGCTTGATGCACCATCCAGATCGGCATAGTGGAGCCA
GTGCTGAGGTT CAGAAGGAGGAGGAGAAGAAGTTCAAGGAAGTTGGAGAG
CCTTACTATCCTCTGTACCTCAAGAAAAAGACTCGCTATGACAGTGGACAG
GACCTAGATGAGGAGGGCATGAATATGGGTGATTTGATCCAACAAATATCT
TCAAGGCATTCTTGGCGGTCTGGCGGCTCAGCTTGAAGCATCTGGTCCA
GGGAATTCTTTCAATTGGCTAATGAAGGGCAACCACCCAGAACCCAG
AAAATGCAGATTCACTCAGTTAATCTGAATGTGGAAACAGTTCACCTCCTC
CCTTCATCACGTCTCGTGTGCTAGAGCAGTTCTGTTCTCAGTTGGATGCC
CTGTGTCTCTGTGAGTGGGGTGGAGCAAAGGGAACCAATGCCGAAGACCGAG
GGCAGGGGAGGGAGGCAGGGGGTGGACAGGGAGGCAGTTGTGAATTGT
TTTACTGTTAAC TTTATTAAAAAGAAAAAAAAAAAAAA

FIGURE 14 A

hMLF Protein 268 amino acids

MFRMLNSSFEDDPFFSESILAHRENMRQMIRSFSEPGRDLLSISDGRGRAHNRRG
HNDGEDSLTHTDVSSQTMDQMVSNMRNYMQKLERNFGQLSVDPNGHSFCSSS
VMTYSKIGDEPPKVFQASTQTRRAPGGIKETRKAMRDSDSGLEKMAIGHHIHDR
AHVIKKSKNKKTGDEEVNQEFINMNESDAHAFDEEWQSEVLKYKPGRHNLGNT
RMRSVGHENPGSRELKRREKPQQSPAIEHGRRSNVLDKLHKGSSVKSNNK

FIGURE 14B

hMLF cDNA 1116 base pairs

GTTATGTGTTCCCGTCCGTACTGGAGGCTAGCTCTTCGCGGCCGCCGAG
TTAACATCGTTTCCAATCTGTCGCGGCTGCCGCCACCCAAAGACAGAGCCA
GAATGTTCAGGATGCTGAACAGCAGTTGAGGATGACCCCTTCTCTGAG
TCCATTCTGCACACCGAGAAAATATGCGACAGATGATAAGAAGTTTCTG
AACCCCTTCCAAGAGACTTGCTCAGTATCTGATGGTAGAGGGAGAGCTCA
TAATCGTAGAGGACATAATGATGGTAAGATTCTTGACTCATACAGATGTC
AGCTCTTCCAGACCATGGACCAAATGGTCAAATATGAGAAACTATATGC
AGAAATTAGAAAGAAACTCGGTCAACTTCAGTGGATCCAAATGGACATT
ATTTGTTCTCCTCAGTTATGACTTATTCCAAAATAGGAGATGAACCGCAA
AGGTTTTCAGGCCTCAACTCAAACCTCGTCAGCTCCAGGAGGAATAAAGGA
AACCAGGAAAGCAATGAGAGATTCTGACAGTGGACTAGAAAAAAATGGCTAT
TGGTCATCATATCCATGACCGAGCTCATGTCATTAAAAAGTCAAAGAACAAAG
AAGACTGGAGATGAAGAGGGCAACCAGGAGTTCATCAATATGAATGAAAGC
GATGCTCATGCTTGTGAGGAGTGGCAAAGTGAGGTTTGAAGTACAAAC
CAGGACGACACAATCTAGGAAACACTAGAATGAGAAGTGTGGCCATGAGA
ATCCTGGCTCCCGAGAACTAAAAGAAGGGAGAAACCTCAACAAAGTCCAGC
CATTGAACATGGAAGGAGATCAAATGTTGGGGACAAACTCCACATCAA
GGCTCATCTGTGAAAAGCAACAAAAAAATAGCCATGCATTGATTGTT
TAGTTTGATTGTTAACAGTTAGTAATGGTGTGGTAATAAGCATAAGAC
CAATCTCTGCTGTTAAATCAGTTCTGTCCTGGCAACTTCTGATATCTG
AATGTTCATGAAGGTCTAGCTTATATTGTCCTCTTAGGAATAAAATT
GATTTCAACAAAAAAA

FIGURE 15

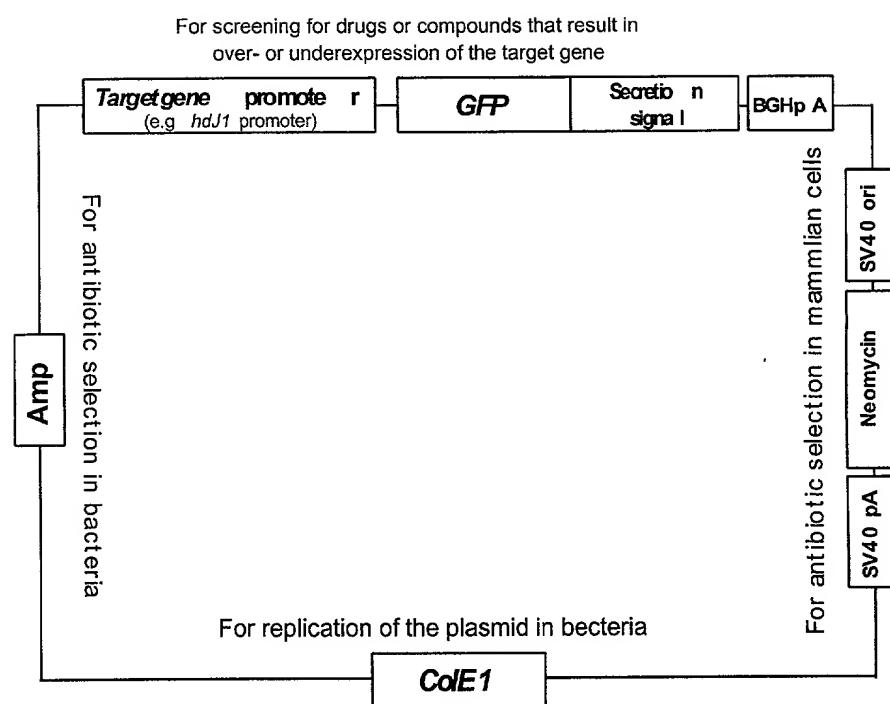


FIGURE 16

dHDJ1 5' region, 24333 base pairs

TTACGGTTATTACTATTACTCTAGTTAATCAAATAAACTGTATAATTCCCTGG
CTTGTACAATAATTGCTAACACGCCGATGCCTCGATCTTTTTTACCGC
TCTCCGTCGTATTCATCATGGTACATATTACATCCAACATACTTATTTTTT
GGGTATTAAACATTGCAATATCGCTCGCCGCCGTTGGTTATGCTCTAT
AAATAAAAGGGGGCGCCGCTAAAATTATAATAAAATTTCATGGGTCTAA
ATCTAGTCTCGAAATCTATGTACAAAGTTGCTGCATGCTGGTTAGGCATAG
GTTCTAACGTATTATTGGGTTGCTTATTCCATTCTGCGCAGTGTGCAGCC
TGTTAGTGTGCTTACGGGTTAACATTAAAAATGAAACATTAGA
GCGGTAAACCTGTTGCTGATTATTGGCGTCATTAAGCGGTATGCCAGCAC
GCGATTGATGCAAGGATAACCGATTCAATGAAATAAAACGAATTGCCAAA
ACAATCTTCATTCTTTTTATCGTACTTAATGATAGCCTAGTTCTA
ATGGGACTGTGTGCTCGGTGAAGGTTGGGATGATTGGGAGGCAACAAT
TATGTTCTAGCTTATAGCTTACAGTCCTACGCCACTCCTATTCTAATATGTT
CATCATCAGCAGTAAAAAACGTTACAAACTCATGCAAATTGAAATCCA
ATAACAAATGCACACGCCGAGTCGCATGGCGTCATCTCTTCCTGACCC
TCGCCTATCCGCATCCAGTTAGGTTGCTGCTGCTGCCGACGGTTGTC
GCCGACTGAAGCCACCGCCGGCGACAGATGTCGTTGCAGGGCTGCTGCTG
CTGGAACCTGGCGCTGCCTGGCTCCGAAGCGGTTGAACTTGAACCTGTTGC
GCTGCTGGAAGTTCTGGCGTAGTTCTGATTGAGAATCGCGGAAATCCTCCA
CCTCCGCCGTTCTGTCAGCGCTCTGGCCCTCGTACTCCTGGAATGGATT
GTACCCGGCGTGTGCTGGCATTGTTCCCTAGCCGAACCGGACTTCA
CCTTCCGCTGACGTCCACGATCCATCTCGTTCTCGTCGTCGATGTCCC
GCTGCCGCTGTCACCGCATCCACCAAGTAGCTACGGAAAACAGAATATCAA
GCATTAGGCTAGAGTCGGACCTTGTGAATGGGAGGCTGGCTGGCTGGCT
GACGCATGCGCTAGTTAATGGAGCTTATGCAGATGAGTACGGTCGCTCGCGA
ACAAGCACTGGGATATGCACATTGTATTGAAATGGGTGAGTGGCTACGG
TTCACGGTTCACTGTAACAGGTTATCAGGCAAAACGGTAACGGCACAAACGGT
TGAATTATGGCGTATCAGGCGGTGAAATGAAAGAAACAACGTGCCGGCCA
GCAGTCAAATCATAAGCTCATTGCACGGAAAACGGATGCCAGTCATCGG
GTGAATTACCTAGGCTCCGGTGCAGTCACTCTCTCCCGCAATGACTTTGCAA
CTCTCTCTACACTTTCACGCTCGTGAACGGAGGACGCGTTGTGGTGACCGC
CCGGTTGGGAACGGATACCAGCAACGCAGCCATCACAGACTATTGGGGTAA
TCGTATTATTGTATTGTTGTGGTATGTGCTTAGTGGGAAAAAGAAG
AAGCGTCGCCTCTGCCGCCACGCTTCTACCTCCTACCGGCCGTGAGAC
GATCCGGATGGGTGCGTCAGCGCTGTCTGTTACCGCCACTGCAATTACG
ACCACATTTACTGTCACTGCCACTAGTCAGTGCCTCGACTGCAACCGA
GCCCTCGACGATATCGCTGCCACTGCGTGAACGACTATCCGTTGCG
CACAAACCAACTCAAAGTCTAAATGAATGGGATAATGTGAAACAAATG
CAAATTACAAACAAGTCGTTAGTAAATCAACTCAATGAAATTGCAATT
GCAACAGCTAAGCGAACGACATAGAAAACAAAAAGAAGACCAAAGAGCCA
GTTAAATAATAAGAATTAGTTAACCCGAAAAAGAGAACCAATTATGTA
CATTTCATCGTATTAAGCCGCAACTGTTATTGAAAGCACAGACCCAAA
GAAAGTGTAAACCAGCATAGATTAGTATCTACGTTAGTGACATGGTCACA
AGGGATAGATAAGCGCTCAAGGTGAATGCCCTCTAAACTCACCTCCTTTC

FIGURE 16 (Continued)

GAGCTCCGGGCTGCCATTCCAAC TGAGCACGGGGAGCCGTATCCACGA
TACGATTGCTTCAGCAGCTATTGATGGTGCCTCCATTGAGGTGGCATTGCT
CTGGTAGCCATTGCCAACCTTGGCTGCTGGTGCAGCTGAGCGCACTGCCGT
TGAGCAACTTTGGCGTTGGCGCTGGCGTGGAGTCCGTGGAGGG
CTTGCTACTCGAGAAGGGATTGCGATGGTCTTGTTCGGCTCGGAATTTC
CCGGCGATCCCTCACCACGACGTAAACATCATCTCGATGGCATCGAC
CTCATCGTTACCGTAACCTCCAGATAACCGTTTCGATTGATGACCGCTG
GCGAGGGTGGCGTCTTGGCGATGGTGGTGCCTTGCTTGAGACTGTGATTGC
TTCTGTGGGTGCCAGCCATTGTTAGCTGAATGCTGGCTCCTCATCGTC
GTCCTCATCACTGTCGCGACTTTTAGGCTCTGAATATCTCGTATGGC
ATCAGTCTTTGTTGCTGTTAACGTAAACCGATGACGAGGCTGAGCCGT
TGGTGTGGCTGCCATTGGTCTTACTGTGACCAATTGGCTGGCCAGACTCTGG
TCCGACTCGCTAGAACATCCTCCCCACTGGACGCTTGCAGGGATTGGTAGAG
GCGCCTCCTCCTCAGATGCTGATTGCTAGGGACAAGACTTTAGTGGC
GTTTGACGGGAGTCTTACTGAATCTTACAGGAGACTTGCCTAGGCTC
CGTATGATTCTCCGTATATTGGGCATGCTCGTAGCTGGCTGTCGGT
TGGGCTTCATCTCATCCTCGATGTCTCATCATCCGAGGAAATTGGCAGATAT
TGTGCTGGTTATTGATTATGGTTGCTGCTGCTGTTGCTGGT
ACTTTGTTACCATTTGCACTGGGAGCTGAAGTAGCTTCGCCTTAGCGTGAG
CGCCAACCAGTGGAGGCTCGCAGTGTCTGAAACTTCCGGTACCCAGCTG
GAGTCCGTTCTGTGGACTTGCTGGTTGCTGAACGGATGGCGGTTT
CTGGGCATTCCATTAGTGAACCGTTGCTCCACCCGCCAGTGAGGAC
CAATGAAACCGCGTTGGTAAGGCGAAGACACAGTCGCCCGGACTGGCGT
TGTGCTGTGCCGTGGTCAAACGCACTCCATTGGGCCTGTTGGCCCGGAC
TGGCAGCCTGTGAGAGGTCCAGTCGAAAAACATTATAGGCATTGTGTT
GCACACACTGTGCATTGCAATTGGCCGCACGTAGCTGTCGTAAGTTGTA
AAGCTGCCGTATCCGTGGAGCCAATGGCCGTGAGTGAACCGCAGTGCTGG
ACGCCCCCAAGTGAGTGACCATCGACACCAAGCGATAGGTGAGCGGTTGAGC
CTGAGCTGCTTGTGAACGGCTGCGTATTGCTCAAATCTATGCGTACTTGA
AGGAAATCTGCTTGGTCAGTTGCTGCCATGGAGAATCGCTCAGCTG
ATACAAAGCGTATTGGGCACGCTCAAAGAGAATTGCTTGTGGCAGATA
CCTGCAAGCGATACGTTAAATAAAATGAACACTACAGAACAAAGGTACAAAG
ACCTACCTTCTTGCATCCCTCGACTTGTAGCCATATCCTCTAGCCGTT
GCGAGAAAAGTGTCCCTCGAAAGCATCCTCAAGGAGTCTGCCTGCGGATG
TCGAGCAACAGATCCTGGAAGTGCTGAAACGTAATGGACACATGGTTGAGC
TCAGACAGCGACCTCGCTGCGCAGATAGCCGCAAAGATCTGCCCAGCG
CGTGGTCTCTTAACCAACTGATCCAGCTTGTAGTTACGAAACCGCATCA
AATACGCCGCTCCATTGGCCTCGACCAGGAAGCGCAGGAACCTGCGCATC
CTCTGGCGACCAACGACCATGTGTTGAGATCTGCTTGTAGCTCGAGTAGA
TGAGGAAGGGTCTGACGGCGACTGATTGCTTGGTGGCCAAAAGTGT
GGTCATGGCGCAAATGATGCAACCGCTGCCAGGTTGGCCACATTGCA
GCCAGATGCGCCTGCTCCGAAACGAGCCAATTGGCCAGGGGGTATGTGCA
GGAGCGCCTGAAGCGTTGAGTTGAGGTAGCAGGTGTTGCCACATTGATCAT
GCCCGTGCCACCTGCCATTGCGCTCCGACTGCTCCAGCCAATGCGTATGT
TCTCCCGCGGATAGAGGACCTCTCGGCTGGCAGCTATTGGGATTGCTT
GTCGGATGCTGATGATTGTTGGTGGTGGTGGACTGATTGTCGGGTG

FIGURE 16 (Continued)

CTCCGCTTGCCTGGCGCCGTATTGTCAGCAAAGGTAAAGAGGACGGTA
GACAGTTAACGACGTGCCACAGGAGAAGGCAGCAGGGAGACAGGAACAGC
TTGTAGAGCAGCCACAGGGCGAACCGTCCACCATTATCACAGTCATAATGC
ATTTATTGGAAGAATTCCCTCTGCAGATTAAGTCACTGATCCCGCTGCTA
TGAAATATAAAATAAAACGAGCAGTGCTCGCTGGAAACTGCTGACACACAA
TCGCGCTTCCATCACCTGTTCGAGTGTGGAAAGGGTACACATTGTTGTAC
CTAGGCACCGGACTGTGCAGCATTAAGATAGCTATTCTATTGAACAAAGAAA
CTTGAACACAAAGTACGCCAAAAAAATTCCAGTACTAGATTGAAAT
ACAATTCTTGAACATCGTACAGAATGTGATATCACCAGATTATCTGAAA
ATATTTCACAGCATCGTAATTCTATGTACCCCTGAATATGTATCTGCAGTT
TTGTTGGAAAGTGTACCAATCGAGGTACTTATCCTGGTACACATATCTCAG
ATATTACCCAGCACTATTGTATCTTGATAACAGCTAGCGTGTGAGCGGGATG
GCGACTGGCAGAAGAAGAAATTAAACTGATAACAGCAAGCGAATGAGAGG
GATGGCGAGTGGTGAAGCAGTCCAAGTGTGCTGCCGACGAATACAGTGGT
CTCGTCTGGCGTAGGGGTTGGGGCGCAGTGTGCCACTGAATTGGC
GCGACCTAACAGTGGTTGTAGGCCAATGCTCCCCCTTTATTGTCTT
GTAACTGTGTCGAGGCATTGACCAGGCCAAAAAAAGAAAAGAAAAGAAA
AGTCGAAACATCGTAAACAGCTCTGGTGTGAGCTTGTGTCACCTCCTG
CTCTGTGTGAATCACTCTCGAGTCTGGCCTTGTGCTCTTTATCAC
GCAAAAGCAGATTGCCGCATTACGCATCTAAAAAAATAAGCAAAGCC
AATAAAAGCACCCTGGGGCTGCCATGTGCCGGGAAAGAGACCGAAGTCA
CGGAGGGGAGCCCTCGTGTCTTTGTCTTTCTCTTCAATTGCCGCTG
GAAATACAGCACGTTTCCGCCACAACCTCTGTGAATCAGAAGTTGGAA
GAGGCCGCTCTGTTGCTGCTGCTGTCACCTTCCAGCTTACTCTTAC
GGCGTTGTAATTGTTGCTTTCCCGTATCCTTGCATTCTGTTACACG
TACACCACCCAAAAACGCCGTACACACGGACACACACGCACGCACAT
ACAGAAGCGCTAAAAAGTACAGGTATGCTGCCGACGTCAGTGCAC
TGCCGACAAAATGCAGGCCGAGCAATAAAATATGTTGCGGAAAAC
ATCACACGTGATTGTTGGAGGGATTCCAAAAGATTGGCAAAACAAAC
GGGACGATAACATAAACATTAAAGTATATACATCTTATATATAATATG
AAGATATAGCACATGGAAAATTGCCAAAAATTGCCACACAAAGAAGAAAAC
ACAGACGAAGGCCGACACGGAAAAAGCCACCTTGTCAAGCAACTTTGGATT
TTACATTGTTGTATCTTACACAGTGTACTCACCAGTCCATTGCC
AAGTTGCCCTGTATTGTTGCCACTAAAGCCATTGATGGCGCTGGATTTC
CCGGCTTGATGACAATATATTGGACTTGAGGTTCTCCAGCACCGATTGTTG
TAGTGGCACCTCCGTATTGATTGGCCATCAGGATGCGTTGGCATT
GGCCACGATGTGATTGCAAGGCTGCCGTGGTGTCTCGCCCTTGGCCT
GGTCAGTCGACGCCGGAGGAGTTGCCAGGATTCGCGCAGCG
CTGCGTTGACGACATTGCCGTTCCGATACGCCATCGAAACGGCATGGC
GATGCTCCGGCTGGGGATTGCGGTGGAATTGAAACGGGTGTGAGGGCGT
GGTGTGGCGTGTGTTGGTGGCCACCCAGTTAGCTAATGCACATGGGC
GTGCGATCCAAAGCAGATACTAGAGATCCTCTGCACAGCCCACACGTC
CAAAACTCTCTACTGCTACGCTACTTCTCGCCCTCTCGAAC
CTTCTGTTCACACACCGACTGCGACACCGACACGCACACTAACGCACTC
GGGAGCACTCTCTTCTGGCTTTCGCGCTGCGATCTCGATCTGTTGGCC
TACTGAGCATTACGATTAAGAACGTTGCTCACAAATTGATCTGTTCAATT

FIGURE 16 (Continued)

TCGTGCGCGGCCAGGCATTTAGAACGAAAAGTCTGCTTCGAAAATAATGG
CAATTCCCTCCCTCGTITTCTTCGACTGCGGATTCTCTTCGCTTCATTTC
GTCATTGGGGATGCCAACTCGCGAGTGGCCAAGTGACCGATAGGCCTCTC
GAAATGTCTAAAGCATTACGATATTACAAAAATGTATTCGATGTTTC
TTAACAAATAAAAAATTGGTTAAATTAAATAAGACATTGTTACCTTGAATAT
GTAAGCAATATCTTATTGAAAGGCTTGCAGCGACATTTTATTATGCCTAC
TATTCAAGTTATAAAATTAAATTTTATAACGGTATTTTACACCTTATCAGCAC
ATATCGATAAGTGTATTGGGAACGACAACCCATCGGCACAATGTTGATGCA
ATTGTTGAGCTAGCCTTCATAATTAGTCGAATCAATCGAGCAGAATGGCTTC
ATCCACAGGTCTCCTGGTGGTCCAACATCAAGCACCTGGCAAATCCCTGC
GAGCCATCGAGAAGTACGTGAATTCACTGTACATCCACCTAAATGTGGCGGG
GTCAACGTCCACGACGTACCAGTCCACCGCCTCCGGTTGGGTCGTCTAA
TCTCGCAGCTGTACGCCAACAGCAGCAGCTATGTGGCAAGCAGTGGACCT
TCGCGTCCTGTCTCTCCCCTACGACCAGGTCCAATGGATCCCTGAAGTTGC
GCCAGCCCCTCGACCTAATCTTCTCGGATGCACATCATCCGGAGCTGTGCGAC
AGGCTTCGCGCGGATCTAACATCAGCAAGCCAACAATCTCCTGGATGACT
CGGTCACTCGGATTAAAGTGCCAGCAGGATGACACCCAGCCCTAAGGT
GTATCCCTCGGTTGTCCTGGCGGAACATTGATCGCATCCATCTGGGACACA
AGATATTCTCACCCAGGCTGTACTCGCGCACCTGCAAGCGTTGGTGTGGC
GTAACCACCTCCGCCATGACGAAGGTAAGACGGCATGAATTGGCAAAATA
AAACGCTTATCTAACGACCATTCTTATCGCTGTGCAGGAAAGACGCTGCC
GGACTTGATTTCGCCGTGGAAGAGCGCATGCCGGCTAAGGGAGTTCTG
GTGGACATAGATGATACGCTGCAGTACGAAATTGTGCCATCGATGATCCCT
TTGGTCCCACGCAAGTGGATCCTGACCTGGACATGATTGTGGTCAGTGGGA
GACGGTGCAGGGAGGGCAGAAGGTCAACGAGGTACGCTCCGTAAGCAACT
GCGCGAGCTGGAGATCTTGTATTGACATTGTTGAAAGCAACGTGCATGAT
GGCATCCACGAGACCAAGGTCAAGCTCGAGTAACACACGCATCGATCTGCTGG
GAACCCGCTGGAGAAGGCCGGAGCCACGACCACAGCTCCGCCGCCCCCTA
CATTATTGGACTCACTGGCGCATCGCATCTGGCAAGAGCAAGATGGCGAG
AGATTGGCAACATGGCGCCACGTGATCGACTGCGATAAGGTGGCGACG
ATGTTACGAACCTGGTCAGTTGTGCTACACCGAATTGTGCAGCACTCGGA
CAGGGTATTGTTCAAGACGATGGTCATCGATCGCTGCCAAGCTGGACCCCT
GGTGGTGCCTGAGCAGTGCAGACTCAACGGCATTGTCTGGCC
GAACCTATTGCGGAGGTTAACAGGCGGCTGGATGCACTGCGTCCCAGGCG
ACGTGCCCGTGTGGTGGTCTGGAGGCAGCGGTGCTGCGAGCGGGCTG
GGAGACCAATTGCCATGAGGTGTGGCCATGATTGTGCCACCGATGAGGCT
GTGCGCGGATTATTGAGCGAACAGTGTGAGCGAAGTGGAGGCCAAAAG
CGACTGGCCAGTCAGGTGCCAATTCTGAGATCGTGGCCAAGTCGCATGTGA
TATTAGTCGAATGGGATCACGAATTCAACCCAGAAACAGGGAGCGTGC
GTGGAAAATGCTACCAAGGAACACTGGACTCTTACCAAGAGCAGCCTTAACCC
GATGGATATTAGATTCTTGTGATCCTTATTTGTATGATTGATGCTGGACG
TTGTTGTATATTGTTAGTTGTAAGTCCAAAGTGTGAAAAGAAATGCTGGACG
TCATTGGGAAAAACGCTGAAATTCAATGGAACCTTAGTGGCTCTGCC
TTCTGCCAGCCACTCGCTGAAGTCGTTCATCTGGTGGTCATGATGGGGGA
ACCGATGAAGCCGATATAATCAATCTCGTCACATGCCACCTGACTGATTGT
TCTTCACGAAGATTGGATGTTGCACATTCTGGAACATTGACGTAGCGCAGA

FIGURE 16 (Continued)

TTCACGGGCACTCCACTCTCCAGCTCCTCTGAGCCAGGCTGAAAATGGATT
GAACAGTGAGAAGAGCTAACGCCATAGAGAAGGCAATAGCTACCTTAGA
TCCTGCACACTGTTCATGGACTCGGCCATGTCAAAGTCAATCGTGCAGGGCTG
GTTAACAGCTCACATCCTGGGACCCAGGTGCGAAGGTGCCTGAAC
TTCAAAGAGTGGATCTCACAGCCTGATTAAGGTGATGGACAGGATGAGCT
GCTCATCGCAATCGGACTGCAGGTAGCCACCAGGCGGAGGCCAGGGCGTGCTT
TAAGTTGTGGTCATCAGCTCGTTGAGGCACTCGACTCCTGCTCGAAATAA
ATGTATTCACTGTTCCATCTGTAAGAAGGATTAGGGATTATTTGGAACATTTC
CAAATACTGCACTATATTACCAATCCCTGCCGTAACTCTGCCCTCCTC
GCCACCGGATGTACCGATGTGCTCCTGGATCTGGCCTCGAGCCCATTGACGT
CCGCACCCCTGGACCGCATCGATCTGGTCCTGTTCCGTAGAAGATGAATGTT
GGCATGGCCGAAACGCCCTGTCAGCAGCCGTGTCCTGGCAGTGTCCACAT
CCACTTCAGGAAGATGGCCTTGGGTACTTGTGGAAACGTCTCGAAGATG
GGCGCAATCCGCTTGAGGGACCACACCACGAAGCTGTGAAGTCCACCA
CCAATTGAATGCCGCTGGCCAACCTCCGCTGGAAAGTGGACTCGTCGTT
GATCACCGCAGGGACATGGTGTAGGATTAGGTTCTATTAAATTGAGCTTT
GTTTCGGCAGCGAATTGGATTAAAGCAAGTAAATGTTATTAAACGTTCAA
TGCAAATTGTTTGTAAAGATGACTTGTAAATATGCATTAGTCAAATTGCGT
GCTAAGAAAAATACCGAATGCGGTATTCCACAAGCGGTACACTGTGATGGT
ATCGATATTGAGCTTTGACTTCCTATTAGGGACCATTTATGTGTA
ATAGAAAAAAACCGAAACTTAATATTAAACTTTATTGAAATATTAGTGG
TTACAATATGAAAATGAAATATTCTCATTGATATAGCTCAAAGTGTAA
TTTAAAATTCAATTCACTGTTACGACTAGCAATCTACGCTTCACGCTCATCTT
AAGCTTACCGCCCATTGCCAGGGTTGTCAAGGCGAATGAGCGTCCACCA
TACACGCCACTGGAACCTTCGATACCTGCGCTGCGCTGGCACACGTTCA
ACCTCGTGGTGTTCAGTCGGTCGCAATTCAATTAGTCGCCATTAAAATT
ATTAGAGTCAAGTACAATGGCAGATGTGGAAAAGGAGCCCGAGAACCAT
CGCCGAGGATTGGTGGTGAACAGTATAAGTTGGCCGGCGAAATCGTCAAC
AGTGAGTATTCCCTGGCCGGAAACAGCGAACGCTGGCGATTCTGGAGTCG
CTGCTACGTGGCGCTTACACAATGCACCGAATGCCCTTCCCTGTGCGCCA
CGCGTTGGTTAATCTGCCTATTCTGGACTCTGTCTGCTGTTAATTAGAA
ACCCCTCAAGGCGGTAAATTGGACTCTCGTGGTGTGCTCTCCGTCCGGAGA
TCTGCACCCAGGGCGACAATCAGCTCACCGAGGAGACCGGAAAGTAAGTG
GTGGCCACCTGGCGGTCAATTGCGCCAATTCAATGTCGAATGATTAAGACTTA
CACCTTGAGGGTTCCCGATGGCGAGCCATGTGCTGTGCGGGCTGGGATC
ACCTCGTGGTCGCCAGGGCGACCGCGGGACTCCAATGCTCCACGTGCCCG
TTGTGTGCTCTCCAAAAGGTCCCAGGGATTACAGATTATGAGATCTGAGGA
CACACCGCGCACTATCATTGATATAGTACAACGAACAAGCAATCTAATGC
TTTATCGATCTTCAAAACAGGTATACAAAAAGGAGAAAGACCTGAAGAA
GGGCATTGCCCTTCCACCTGTCTGTCGTCAACAACTGTGTGCCACTTCT
CGCCAGCCAAAACGATGCTGACTACACGTTAAAGGCCGGTGTGGTCAA
AATGTAAGTGAACCTCTATTCCACATATACCGCCACTAAATACGTAACATT
TCTTTCTACAGCGATCTGGGTGCCACATTGATGGTTCTGCGCTGGCC
CTCACACAATTGTGGTAGGCGCTGCTGCGGATCAGAAGATCAGTGGTCG
GCCGATGTCATCCTCGCCGCTACTGGCTGTCCAGGCTGCCATTACGTCTG
TCAAGTCCGGCGCCAATGTGAGTCCTCCATTACTCTAGGTAATCCTCCGTTA

FIGURE 16 (Continued)

ATCCCTGCAAGAACGGATTGTCTGCCCGATTCTCCAGCGACTGAACATCTC
AACACTTGAAAGATCAGCTGTGGCAGCTGTAATTGCCCTGGCTTATTATTC
AGGACTGGAGGCTTCTGTCAAGTTTACAGGTTATTCTCTGCAGGCAA
CGGATTGACTGCGCTCAAACACTGACACAGATCAGCTAACACCTGCGGATA
GAAACTGTGTCATTCTGTGAACAGTCAGTAACTCCATAGAAGTGGTGG
TCTTAAATTGTCCACATCTCCAGTTATAGATATGTCGGAATTGTAATCTGC
AGGCAACGGATTGTCTGCCTTAACCTCGTGGCTCAGCACAGCTAACGTCT
GCAGAGATCAACAGTGTGATTCTGTGAACAGTTAGATACTTGAA
ATGTTGGTCTTAAAGTTGTCCACAATCGCAATGATAATGCCGATCAGTTAT
TGTATTTCGTTATCTATAGTATACTATGATATTGATTAAGATTAGTCAAA
GGGAATTGGAATGTTCTTATCTGCTTGAACATTTCCATTATTCA
TACTTAATTTATGTTCAATTCTGTATCCTTACAGAACTACTCCCTACCGA
TGCAGTGCAACAAATCAGCGAGTCGTATAAGTGCAAGGCCATTGAGGGCATG
CTCAGTCACGAGCTGAAGCAGTCAAAATTGACGGCGAGAAGACGATCATAAC
AGAACCCCCAGCGAGGCGCAGCGCAAGGAGCATGAGAAGTGCACCTCGAAA
CGTACGAGGTGTATGCCATCGATGTTATCGTCAAGTACCGGCGAAGGAGTGGT
TAGTAATCCATCAATAGACACTACATCTCCACTAATTGTTGATGATTAAAA
ACACCGCCTTGAGGCTGACTTGCTGGAATGCGGTGTTGCGAGAGTGA
CTTGTGCTCGCGTTTTTATACTAAAATGCGGCACGTGCAGACACCAAG
TTCCGGCTGGCTGTTGTCGAAGATTGCAAGATTGAGATCTGAGAACGCC
AAATTAAAGCTGGATCCTGGATCATCGCAGCCAGAGCATTATTGCTAACATTA
TTCGTATTCGTTGCAGGGACGCGAAAAGGACACCAAGGTCTCAATTACAAG
AAGTCTGAGGAGAACTACATGCTCAAGATGAAGGCGCCGTGCTCTGCTGG
CAGAGGTAAAACCAAGTACGGAAACATGCCATTCAACATCCGAGCTTCGA
GGAGGAGACCAAGGCCGCATGGGAGTTGAGTGCCTGGCCACAAGAT
GATTGAGCCCTCCAAGTGCTGTACGAGAACGCACTGTAAGTGTGATGCATAT
TATTATTAAATCCTATTCCCTATTATGCGAGTTGGCAGAACTTAATTCCGGACC
TGGTACACCTCGGGTGCTAAGTGCGGCCAGACATTGCGCAAGAACAAATTC
CAGGCATTGCGTCTTCAGCAGTTGCCTCAGTGTGGCCTCTGTCTGAACATGG
CACTGTACAATCGTATCCAATCTATTAAACCTGTTCTTACTTAAAGTCGAAATTAGC
TAATTAGAGACTAAACTAGTTGAGCAACCTTATAAAAGTCGAAATTAGC
CGGAAGTAATAGCAAAGTAAACAATCCTTTCTTATCTGCATTACAGCCG
AGATTGTGGCGCAGTTAACGACACGGTTCTGCTCATGCCTAACGGCGTCAA
CTTGGTCACCGGCATCCCATTGAGGGAGAACATGTGAGCGAGTACAGT
GTTGCGCAGGAGGAGCTCAAGGTAAGCTGCAACAATTCCCTGTATTACGA
TGCCTACTCAATGAAATCTCAACTTTGCAAGACTCTGCTCGCGAGCCTTG
GGTGCCTGTGAAGGGCAAGGGTAAGGGCAAGAAGGCAACAGCTGGGGCGCG
ACAAAGGTGGAAACGGCGCCGGCGTGGAGACCAAGGCATAGACCGAGCCCG
CTGATGATGCCGACCGCCAAGCCATCAACGGAAACACAATGTGAACAAAT
TGCCTGCCAACGCTGCCTCACAGATTACTATCGAATTGTTGCGTAA
TTAGAGGACCTTGTACAACAGAACAGGACAGAAGAGAAGACGGCAACAA
TTTGAGGACATTTCCCCAGAAATCCTCCATCCATCAACAAAGGCGGGCGGT
CGGTCGGTCCCGCCAACCTTACCTTATTCCCTTACTATAAGCTGCCTT
CGTTATCGGTCTGTCACATCATCGCAACGAAAAAGCAAAGCAAGAACTG
TCATCAAATTGTAACAATTAAACGCTAAAGTAAATTAAACATTCTAT
GAAACGTTATTAAACGCTGCGTAGTAGGTATTAAATAAAATTAAACATTCTAT

FIGURE 16 (Continued)

AAAACAGCCATAAATGCCAACGATTTCATTATTACTTCCGCTGGCG
CCCAATTAAATCGATTCGATACGCTCTCATTCTAATAATGCACTTGCG
AGTTGTGTTATTTATACGTTAATTAGTTGATGTCACATTCACATTAT
ACAATTGTAATTAGATTCTGCCTTGTTATTAAATTACAGTCTCA
TCTTGAACCTCTGTATTACGAAAGTTGCAAGAATAACTCGTTATGTTAAAC
GTCACCTAGTGCTGTGCTACTGGCCACCCCAGTTGTCATCCCAGATCCAA
TCCCAACAAGACCAGACCAATTGATGCCGTATACGGCGACTTGCCCCAACT
CGCTGACCTCTCCCTGCGTCAAACAAAATAAGAACAAACAAAAACGCAA
TTGCTGCGGATGAAGTATAGAAAACACGAGCAGCAGTCAGACGACAAAG
ATATGTGGCCGGTGTCAAAAGAGGATCTGGGATTAAATGGTCTGCCGTGCG
TTACATACATGGTTGGTGTACTTTTTTTGTTATGATGCCGCCGACTG
TTTCTACTCGCCAGACTAATTATTGACATGCACGTCATCGGTGCGGAGGCG
GTCACGTTGCTCGACTCTCCGGAGAGTCCAGGTAAATCTCAAGGCACGTC
CCGGCGCTGCGCATACCGCGTGGTGGACACGCAGCCCACCCGATCCAGTC
GCCTCTCCCTGGCGTTATCAGGCGTCGCTCCAGCGTCAGCTCTCGCG
AGGTACCGTCCTATCTCTGTGAATTCAATTGGTAGTCTAGGAACACTGAAC
CACTGCTCCACGCTTACTGTACAGGTAGATGTTCTGTCTGTGCTATT
AGCGGATATTGTCAGGGTGGTGGGACGGAGTACCAACTGCTGGACTCG
TAACACTTAACGCTGATATGCTTGTGCAAGGGAAATTGCTGTTCAACTGCAG
AGAAGACCAATTAGATCAATATACACAGTAGAACGCAATTACGAACCTTC
ATATAGCTCAGTTGTCTATTGGATGTCGCTGATCTGTGATAATGAAAGTCT
GATTGCGTGCCTCTGCAGAAGATACGAGTTGATTACTGCTTACAGGGCA
ATATACAGATTAACTTACGGTCCAAAGTGATTCTGGAAACTTCCAAACTC
CAGTTAGCCGGACACCACCGCTTACAAATAAGTCAGAGAACGTCCTTG
GGCTCGGGTCCACTCCTCTCGCACTTGCACAATGAACACTGAAATGGTGT
GATAAAATCCAAGTTAAGTTTTCTCATCACAGAGACAGGGAAACCCA
CCTCCGCTGCGATGCTGGAGCGCATGTCGAGTAGAGCGTTCATCGCGA
CAGAATTGATGGAATACGTCCGCCCTCAGCCAGATGCGACAGTTGCA
CAGACAGTGTGGCGTACTGGCGTCTATGCGATGCGAGCTGGCCACCGAGTCC
TTCTGGCCTGCTCCGCCGTGGTGTGGCATTGTAGACCCACTCGCATAACGCA
AGGCAGTTGGACGTCTCGTTATCAATGTCGCCAGTCGAGGAAGTGAATC
TTGGCCTTGAACTCGTCCGGCTCAGCGTCTACCCAACTCCACCGTCAGTGT
TTCGCCTCTATGAGATGTACCAACGAGTTGTTCTGGTTGTCAGACAGATT
TATCGTGTGTTCCGCTGCAGTTAAAGTGGCGGGCACTGGATCAGCTGC
TCGATGTGTTCTAAAGGCCCTACCGCATGTGTGCCACCAGCAGCTT
ATAGGCACCGCGTGGGCTACGAGCTGAGCCTTCGTCGACTGGTACCA
GAACGGAAACCGGTGCCAACACATCCACCGACTCGACCTTGCGTAGA
AGAAGTGGTTGGTGTGGTGGCAGGAGCAGCGGATCCACCATCGGCC
CGCATAACTGCCGTTCCATTGCACTAGGCATGAACGCGCATCGTGGCATCGT
GTGAGGCTGCTCGCCTCCGGACTAGACAGCTGCGCGAGTGACTGGTGG
ACTGACTTGGCTGTCGCCTCCACCGCGATGTGCCATGTTGTCGCTCCACCA
GAGTTCGGTCTCCCGCTCAGACTACTGTCCTCCAGTTGGACTCGTGGCG
TGGCTGGACTGGGTTGTGACATGGGTTCCACTAGATCTCGCTGTAGCGCCT
CCAATCGTAGTCGTTGCTGGATGACATGTGACCGAGGTGCCACGCCATT
TTGCAAGCATCCACAACATCACCCCCGCTGGCGACTGAAAGGACAGCCAAA
ATTAACCTTAGTTATAACCCAACAGCTGTATAACCTACCTCGACTCAAA

FIGURE 16 (Continued)

CGGTGGGCACACCGAGCATCTCAAGGGTCGGCATCCGTGTTGGGTACGTT
CAAGTAGAAGTATGTTATGGACTTAAACTGGGTGTTGGCCATGTTCTGGAGA
TGTTGCAGGGCCTCCGGCGTCGGATCGGATCGTAGGACACGAAAGCCTCG
GCACCGTGGCGCGCACCGTGGCGAGTAGGAACCTGCTGCTCGCTAATGTCAA
ACGGAGGGCGATCGAGCGCGAACGGACGTCGCTGGCTCCCGCTCCCGCGCT
GCTGAGTAAACCAGGAAGGGACCGTCCATGCCATGGTCGATAGATCTACCT
TAAACACATACCAAGTAATGCCGTTGGCGGATAGACCTCAAACCTTGGTC
CTCGGCCCGGTACTCCAGCAGGAAGTCGAGACTGTAGTTCTGCGCCGCGC
AGTCGGTCAAAGCTGGTCTGTGCAACTCTCCAGGGACTGAATAATCGTGT
CCATCGAGGAGTTGTAAGCCACCAACGACAGCGGGAAAGCGGCGGAATT
GTTCCACATTCAACATCTCATAGGCCGACATGAGGACCAAGTTGATGTTGAA
ACTCTGCGATACGTACACTCTGGTAATCTTCATCTTCTTCAGGGAGGGATTAT
AGAAGTAGACGCGCGGCTTGTACAACATCCGAAGAGCCAAGTCCGTAAACCGT
GATATGCCTGCCAACCGGGACACGCGCGTCTCCTCCGAGTGCAGCTTG
TAGCAGCGTTTGATGTGCTCAGGAAAGTCGGCCACCTGGCGACTAGCTC
GTTCTTTGGCATCCACCTGCCGGTACATCAACATGTATGCATTGGTGCTGG
AGGTGTAGGACTGGAGTAGTAGCTCCGTTGGGTCCGCCAACGAAACGTTG
GATGTCCTCTGGGTGATCTATGGATAGATAGTCGTTCAATATTTCTCAAGT
TATGAATGTGTTGCAAAACCTACACTAGTCACGTTCTGATCGTTAAAACAG
AACCACTCGTTGTTGCGAAGTCCTTAATATAAGCATAGTAGTGTCCGCCGA
AGCGCTGCCTGAATGAATCATGATGGCGAACAGTCGAGAGATACGGACCG
GATCCTTGCTTGGCGCTTGTGCTGGTCTGCTCATGTCGATGCCTCATCC
TCGTCGTTCAGATCGTTGCTGACTAGAGCTCGCTGTGGTCACACGCC
GCTGCTCAAATTATCGTCCTCATGGCGGATCCACTATCCGCCGTGCTGCAAT
CGTCCACGGTGCCTGAGCTGAGAGTTGCTCACCGCTGTTCCACTTCGG
TTAATGAACGTGTTCAGGTTGAGCGCTGAGGGAAAGTCACTCTGAAATAGA
GGCGAGCATGGAATTAAATGCTTATGGATTATGGCAAAGAGACTAACCTGT
CGTTAATTGATGCGGTGATGGTCTGGTAGTCAAAGTCAAAGCGTTAAGG
TGCAGCGTGAGGATGTAGGGAAAGGACTAAAGTCAGTCAGTCCCTGTGGCGT
CGCATTTCCTGCACCTCTGCACAGATACTGGTATTGCCATCGAGTGT
CGGGCTGAACGAAGGCACGCAGAGCTCCTCGATGCTGCCGTATGCGGAGCT
GCTTCCAAAGGGCCTCACAGGGAGCGGGATATCTAGAAAGGTGTCCTCGCG
GTCTCTCGGTATTGCACTCCAAACACTGACATAATCATTGTCATCTGCCCTC
GTACAGATTAGAGATGAGATTGCTGCTTAGTGTCTTGAATTGTGCTCCA
GAGCGTCGAACATAACTCGGCACAGTCCTGGATATCGTGCTGCTGCCATGC
CTCCGTCAGTCCCACCCAAAGCTGCGAGTCAGGTCTGTGGTTCTACCGCCG
CTTGGCGAGGTCTGCAAGTTGAGGAAGAGCTTGCAGTGGTATGGTAT
GTTCTGGCCTCGTTGTCATTGTCGAACCTCCAGCGGTACAGAGCATTCTGA
ACTCGGGTGTCAAAAGAGTCGCTGCAAGCTGTTAGATAGCAGGTCA
GGCTGGTTGACCAACAAACATATCCCCTGGACCCAAAGCTGCCTGCCTG
CCTCTGCCTCAGTTCTGTGGTGGCCGAGGACACGAAGTCCGCACCGTTGTG
TTGACTCTGCCATGCTGCAACTCATGCCCTCCATACTTGCAGCAGT
GTTGACAGAGCCGGTACGTACCATCGTCTGTTCAATTGAGATGGTCTG
TCACTCCGGTCACACCCTGACGTCCGAGTCACCGGTGGAGCTCCGTAATC
GTATCCAGGTCCCAGCATTGTCGGACTAGCAGATGCTCCGAGTGCAGGTG
TCATCCGACAATTGTCAGCATCTGAGATGAAAAGATCTGAAGGAGCATCGT

FIGURE 16 (Continued)

CCACCGGTGAGATTAGGTTCTACCTTGTGGATACAGCTGCAGTGCTCAGAG
AGCTGTTGCTTGTCAAGGGTGTCTACTGGTTCGAGTCCAACCTTTGATGGG
TGATGACGGCTTGATTGGACTCAGCAACTCTAAAGTCGGCTAGAAGTGACC
TTGCCGTTTCTTGGCACAGGACTCTCGAAGAAGTGTAGAGTTAT
CTCTGTAGACAGCTCGGGACATTCTCAGGGCTAGCCCTGGGAGCTTTCCG
AACCGGGCTTGGAGATCTTGCTGCAGTCGCTTGATCTTGAAGTCTTCG
GGACTTGATTCCGAACGTGCTGGCTTAGCCAAAGAGTCCTCACTCGTGT
CTTGGCCTTGTGGAGAGGAAGATCCCAGCTGGCTCTTTGTTCT
CACCAACTACCGTCTTCTCTCGCCGGTGGCAGGACTCTGGCCTTCTCG
CCGTCGACTTCATAACCTTTGACTACCAACTTTAATGGTAACCAA
GCGTTGGTCACGTACCATCCAACTGCCGGAGGGCAGCAGGATCAAGTGA
TTCTTCAGCTGGGCTAAAACCAGCCACTTCGTACATCAGCTGAGATTCCAG
GGCATTCAAGATTGACCTAAAGTAAAGGGATTCAATTAGCGGTTATTAGA
ACCTCAAGATGTGAGATACTTACAGATCCTTGTATCGTGTGGCTGCAG
CAACAGCTCGAACTTTCGTACGAGAACTGCGTGCCTAAAGGTCAATCAG
CGTTCACCGTGAAGTGGGAGCGGACACTACGTTGATCTCTTTGCTCCGA
GCCGGGTGTCTGGTCAAAGACCGAGACGGTGCAGTGCTCGCTCTCCTGTCCG
TCATGTCCAGCCCCGGAATAATCAAGTGTGGAGAAAACCTGCAAAA
AGATTGTAGGCAGAACGTTGGCTTACTTATGAATTGTCTGGAGTTCTT
TTATTTTTTATTCTTATTAGAATTAAAAAGGTGACACGACACCTTG
ACGTTTCGGCGGGCCAAGTTCCTGGACATGACGATGCTTCTGGCCATAG
TAAATAAGGAAGAGATGCCAGCCCCAAATTACTGCGAAATCTTCTTGT
GACCCATTGCGAATAAGCGGAGAACCCAAGAAGATTCCGTCCCACCTC
CCGAGCCGAGATATTGACGTGCTCCGGTTGCTTCTCGCGCTTATTGT
ACGGGCCAGCACCAGTGCCTACATATATATATATATAGATAGAT
ATACACATATAGCACGTACACCCAAATCGAGCATCGACTGCCCGAAATCG
ACGCGTACTAACCGCAGGGAAATTCTGAAACAAACCGGCCATCAGAGTT
GCCTCCGGAGGATGCTACGGGAATTATTATTGCCTCCAATGGACTACCAAC
GTCATCATCATCATGACCATAGCTATCACCATGGCGTACCGAATGCAT
AAATTCAGTGCAAATGCGCTCCATGTTAGCTGGCTTCTTGTGGCTCC
CCGCAAGACTCTGTAACGGAAGTGGTGGCTATTATACGAACGAATATCTGGC
GCCTCAATTGGCAGTGCCTACATGCAAGTGGACGGTGGACATATCCATA
TGTACAAATTAAACTTATCGGACATCAGCGTGAACACTGCGAATTATTCTAG
AAACATTGTAGAATTGAAAGATTAAAGGAAAGCAGATGCTGAATATTAGG
CGAAAAGCGATTGAAACTACTCTATAATATGCAGTCAAAATATCATCGATT
GCCTGTCAATTAAATTGTATCTAAATTACTTCTGAATGTCTATTGGCAA
TAATCTTAGTGTACTGCTCAGCATTAAATTGAGTGTGCAAGCAATT
GGGGCCGGGGTATTGCAATGTTTCCAATTCTCTGCACCGAAATAACCACA
AAAAAGACGCCAGTCAGCCAAGATATTGGGTCTCCTCCGAATGGAGGAT
GCACATCCACGATGTGCGATGTGAATGCGCTGCAATTGGCGTCAACACACA
TGTGGATGGTCCAAACACAAACCGCATTGCCGGCAAGGGAGCGAGTGAGA
TGGGGATCCAAAATGCTAATACACGTCGGCCAGCACAAATCAAATAAG
AAACCCATGCTGCTAAAATAAAAGCACACAGCGAGTGGAGGAGCAAGATATAAGAC
CGGAGCGGTGGAAAAAGCACACAGCGAGTGGAGGAGCAAGATATAAGAC
AGCTTGGGAGCGTCTGAATACGCGTATATCTGGCTATTGTGAATGCGAAG
GTTTTGAGAAATTCAAGAGAAGCGCACAGACTGTTGCAATACGTCTATCCTAT

FIGURE 16 (Continued)

ACATCAGAACGGTCAGGCACTTCAACACACATTGGCCCCATCCATCCACTCAA
TATTACATGATGACGATGATCTTGGTCAATGTTGTGGTCGGTATT
ACAGAAACCGATATCGCGAGTTATCTATGCCATATAACAGATCCAATGGGGG
GACGGCGGGAGGGCAACAGTCATGCTGCATATAATTGTGCTATTGGAA
CTATTGGTACTCGAATCTATGTGATCTACAAAAACCATGAGATGTCTGA
GATATGACTGCTGAGTGCCGGAAATTGTAGGATTCTGATTCCGATCATATA
ATGCATTCTCGAACAGAAAATCTCATTACGAAATGCTTCTATTCTAGGCG
TCGCACAACTTAATTGGAGCTTCAATGTTGTGAATAAGTGTATATAT
CCGTGGTCTATATATGCAACGGATTGGTGAAGTTACCGTCTGTGCGGAA
CTGAGTGTGCCAACATTTCCGAACAGAACAGCACCACCGAACGGCACGG
CATAGTTACCGCGTGTACTGGCCGCTTAGGATGCCATGCCGATTCCGATTGC
GATCCGAAGATAACACCACCCGATCTGGCGCCGATCTTGGCGAAGCGAGCT
ACGTGTTAAGTTCTGGCGTGTACTATAACAATGAGAACAGTTACTTA
TCTGGCTTACACTCAATAGGAAAACAATACTTTATATAGCTTCTATAACTT
CGGGGTGCGATAAGAACATGAATAACAGATAACACGGATTGCAACAGTACCCA
AGCCACTTGTAAACAAATAACAGGATAATGGGGAGTAATGTAAGCTATT
GACTGGGTTACAATCAGGGTCTGATAACAAATCAAACATTGTCCAGTTGCCCT
TTGCGAATATCAATGACCACTCACGAGTTGCAACTGATAACGATTATCGCCG
CACAATGCAGTGGGTGGGTATTCACTGGGGGAACCTTGGGTCCCTAGAA
CCCAGACGGATTACTCAATGAATAAGGCATATGGCGATATGTTGGGTTACAGCGAA
AGTGTATTAAATGTCGACCGTATGCTCTTCGATGTGCCAGCTCTATTG
GGGAATGAATGACTATTTATGGGTCTGCCGCTGCTACAATGCTGCATT
GCTGCAGTGGGACATCCTTGAACAGCGCCATGCCAAAGGATATTCTTGT
GGAAGGGGGGGGGGGCAAGGGTAAGGGTCACATTGCTTGCAGAAC
TTCCAGCGATGGGGCGGTGAACGGTGGCGGGCGATGGTCAAGGCTTCGA
CTGTGGAACGTGACACGATATGTCGGCCGGAGTTGGCCAAAAAGTGGCC
CCAATGGTTGCCTCGCGCTGGCAATTAGTCCCTAGCAAGGCGCTCCATAT
TTGCAAAAATTGCGGGCGCTTGTGTTCTCTGTATGTGATGTGCT
TGTGTACCTCCGTCTCACTCACCTCAAGTGTGTGTATGAAAATCTG
CGGTATACGGCTCGCTTGTGAGTGTGGTTCTGGCTCTACTCTCCGA
TGATCCTGCTCCTCCGGCTTAATCCGGCTGCTCGGCTGCTCCTGCGTCC
GACTGCGCTAGAAATTGCTTAAACAGAGCCTCGACGGTCATTAACT
GTTTTGTGTTCCGTTGCTGTTACAGACGTGCTCGTCCGGTGTGAC
TGCCCGCCGCTGACTGAAAATACTAAACGCATTGCAAGCTGGCAATGCC
AAGTCTGGTCTACGGTACACTGGCAAAGTTAAAATTATTAACT
CTTCAGTTACTTCGTTGGCTGAATATTACACTAAGAATTCAATTGACAC
TTGCAATTATACATTGTATATTATAATTATATTGTATTATTTATATC
ATATAAGATATTATATCTATTGATCTTGTGATTATAAGCTTTGGTGAAC
AATATAAGTGAACCTTCTCCATCACCTCTATCTTACAATATGCTTAC
TCGTCATAACGTTCTATTCAAATATTCAATATTCAAAGAAATATT
GTTTATTCTGTGTTAAGCAATCTGACCCCTGAGAAGAATCCCTA
TAATATTAAACAAATGTATCCTAAACAGATCGATCTATCTCGCAGACTT
ACACGAAACATTCCAGAACCGATAGTTATGCGATATGAGATTAAAGGA
GTACTTCCGCATTGCCATCACAGTCACGCTTCCGGATTGCAATCA
AATAAGCGCTAATAATAATCGTAAAAGCATAAGAAGCATATAAGAAGAGT
CACCGCCAAAAGCATGCACAAATATATAATGGGAGCGATTAAAAACA

FIGURE 16 (Continued)

GTGCACTGTGTTAAAACATCGACAGCTATCGGTTAGCATATCGATATTGACA
TTCGCAGTCAAACGTTTCGAGATAACAACCCTAAAATCCGAGAAGCATCCAG
AAATTCGACGTAGACGAGGGCGAACCTATAAAATGAGGTTGACGCACGAAT
CCCGCTCATTATTAAACAAAATTTGAAGAGAAAGAAACTCTGAAGTAGGGT
GTGTTTTAGTGCGCAAGCACATTGGTGGATAAAG

FIGURE 17

dTPR2 5' region, 13015 base pairs

AGACAAAGACAGCGCTGACTCGACTTCGTATTCAATTGTTAAATGACA
TGCAAATGTACGAATGACATGGCATTGCCAAGGGTTTGAAGGGGGGCC
AGATCCAAGGGCAGGTCTCAGGGAAATGTTCCAGGCTAATTGTGGGTTT
ACGCCCTGTACTTCTCCAAATGATCAAGTACGTCAATTAAATGGAAGGCCACTGA
CAATTGGAATCGTAAATTATACAGCACAAACTAGATTGTTGAGTGCTCTCA
ATGTAGGCTAATATTAGATTCTGCCTGAATTAAAATTATTGTAATACGTAT
TATAATGCATTGTACCCAAATTGACAGACTTAAGCAGTTCTCTAACATAAT
TGGCATCATTGGCAAAGAGAAAATAATTAAATTGGCAGCATTGCCAGAAAA
AACTCTTCTCTAAATTGCTGATTGAATGTTGAGTTGAGAATGTTGTA
AATAGTGTAGTATTGTAACACACGACATTTCAAATATTAAATGAAAATC
ACATGGTAATTAGCAATTGGGTGGCCTCTTCCTCCCCAAGCCAAGCCA
TATAATTTCAGCCAGCTACTTGCATTTCCTCCCCATGACCAACAACAGCCC
CATATGTGCAGTGCATTAAATGCAGATTCTTGGCAATTGTTTGCTACTTTG
TTTTCTCACTCACTCAATTCAATTGGCGTGCTAATAACTCATTAGTTC
GCAACAAAAAAACAAAAACGAACAGCGGGCCACAAAAAAATGTAGCTACAA
ACATGGCACACCAACAATGGATTGGATGGCTAACCAAGATGCCCAACTTC
CCTTCCATCAATTGCAATATATCGCATCTCATGATGCTGAGAGAATACTCG
TACTCAACTATGCCACTTATATGAACACTGTGTGCAGTTGTTAGGCTT
TGTAATTATTATAAAAATAATTGAACTATTGTTGCCTCATTAGATTGAACA
GTGAGGCAGCCACAATGTTGCTTTGTTATCGGATACACTCAATTAGCTGA
ATTGCAAATGCAAATGGCCCGTATGAAACTCACACCTCGAAAATCATAGA
CTCGAATTATTAGAAATTAAATATAATTATATTGTTCTTCTTTTTTT
GGTTGGTTTTTTTTTTGTTTTGTTCTTGCAACACTTTCCGCCCTC
TCATTTGACAGCCGAGGAGTCGGTGGTCAGTTGATCTCTGATTGTCA
GTCAGTCATTGTGATTAGACATTGACAGTCGCCCTATTGTTGGATGGCAT
AAATTATAGTCTGCTCAACAACAAAGCGCTGCATATGAAATCCACATAATA
AATCAATGTGCTGTCGAATTGTTAAGTTATTGTAATCAATTGAATTCT
CGCCGTACCTCCCCACCCCTCGGGTGGTGGAGATTATGGGAATATTATT
CATTGCTATTGGTTAAATGGCTTTGGGGTTTCCCAGAATATAAGTTA
AAATTAAACGCGGCAATAGGCTTAAGATCATGTAATATTATATTGCCGTA
AACAAATGCTTCTACTTCATTATCATGAGTGTGTTAAAACCTCCACGACTGC
TCTAAACTTAATCTTAAATATTGTTACCCCTTGAAGAAACTAACCAACTTAG
CAAATCCCTCTATTATTGCCCTAAACTCTGCACTATCGAACTCGCTTCCCT
TCCCCGCCATCTCACTCGAACAAATTAAACAACAAATTAAACTGAAATGCA
GTCAAATCAATCGCTGACTTTCAATTGTTCTCTTCCGGCCAAACA
TTTCCACTGGCCCGAGCGTTGCTAGTCCATGGCTTGCATTGGATCGGC
TCGGATCGGTTGGTAAGTCTCGGCGAGTATGGCTTAGTCCAATTAGTGG
AAAGGTGTGCCACCAGCTCGGTACAACACGTTGCTGTGGCTATTGGAGT
TCGGCTTGCCTCGCTGGCTTGAGCCGTTGGTCGGTGGCTTAAACGC
CGTTTAGCCAAGTTAGGTGAAAATGCCAAGGGAGTGGAGTGGAGGAC
GAACTGTCAACTGTGATCAAAATCAATTGTTGCCATTGCAAACCAAAATTG
ACTGAGCCAAGTCAGTGCAGTCACACAAAAATGCTGACAAAATTATACCAT
AACCCATGAAATGTCAGTGTCAATAATTGTAATTATGAGAGCATTGAGCT
TGAGTACATAAAAAAAAGTTATATATTAAAAAAATCATTATTAGTTGGC

FIGURE 17 (Continued)

TGCCATTGGAGAAGCCCCAAAAAAGGCAAACAAATATAATAAAAAATTATT
GCAACGTAAGTTGATTGAACAAAAGGCGTATAACAATTGGATGAGCTCAA
GAGTGTAGAGTGAAAATGTGAGGATCATTGTCGCAACCAACTAACAGA
GGTCGTCTCTAACATTTCAAAAAAATTACATAACTTTAAATTGATTCA
GTTTATTGTAAGTGAGAAGCCTATTCTAACCATAAATTCTGCACGTTAAG
AGTATTCCCTTCATATCGTATCTACAAAATCAATCCAACACACCTGTTCA
TCTACCGTTAACACCCTAACGCCCCCATTCTTATCGAAAATATAGCC
CTTTTCACGCTCTATTATAGCATTACATTCTTCTTTTTGCACCTTT
AGCTGGCATATCCTTCGACTCCGCCATTGAGGCTCGCCCAATTCCGTTT
CGAGTTAATTAAATAAAACAAATTCTTCGCTCTAAAACCTCAAGT
GTATCGATACGATGCGTTCTTTCTCGTTAAATAAAATAACCCAAA
AAAAAAACCAAAAAGTAGGAGGAGAAAAGTTATTGCCATAGTTTTT
ATTATACTTGTGTGTTACCTTCTGGCTTGATCGATAGGCATCTGCAATT
AAAAAGAGAAGAAGAAGAGACAAGTACTGCAACATAACGGAAGGAAACACGG
TAAGCTTAATACGAAAAACAAGTACTGCAACATAACGGAAGGAAACACGG
CTTAAATTGGGGCACAAATGCTGAAAGGGAAAGTTTCTATTGACGGGTCG
TTCTGACGGACTTGCAATTGGGGCAAGCGGGTGTGAAATGCACACGCC
CCGAGAACCCCCCTTCCACCCCCCTGGACCCCTTATCCAGCCCCTGGCC
AAAAACAATTGTAATTATCCACAGAGAGCGCTGCCTCAGCGGTTTCGATT
TCCCCCTTCGCTCGCTCTCCAACTTGTGTTCAATTAGCGAAAACCTTTCAA
CCTAATAATAGGTTAACCGCATTAAACCGTCTCATGTTCGGTCCGGTTC
GGTTTCAAAACCGGGATCGTACTTAGACTGGGTCTCCTTATTCTGTTCTG
GCTCTGTACAACCTTCTATTGAGAAAATGTAAGTACTAGTTTCTAGCAAC
GGAATACAATTAAATCCAATCCAATAGTTAACGAAATACAAATGATATT
ACTACCATTCTATTTCGTTAACCGTAAATTGACTTATTGGCTGGATTACT
TTTCAAAATATATGTTATCAATAAGACACAAACCTACTTTCTAGCTATTAA
CATAGTTAAAAAAAAAAACTAATAAAATTACGTGAATCTAAATT
TAAACCGATATCCAAGAAGATCTCAATTGGCTGTACTCAGTCTCTG
ACAAAGCGATGTGCACTTGGAGCACACTCCATACATGTGGCTAGCCCT
TTCCATAATTAACTAGATGGTTCCATCGACTCATTGTTGTCAGCGGCCA
GTTCAACCCGTTCTCACTGCAACCGAGAACTGTAACACACAAAAACCCAGG
ACTCTACATTGGCTAAAAAAATAAGAACAGAACAGAACCAAAACCAAAA
AAGAAGGGATATTGAAATACAAGGTTGTAATCGTTGACTGTTGATGTCTC
AATGCATGGCAGTCAGTTAGTAATGTTTCAAAATCTTCAGGCAGGAG
ATGTTAAATATCCATGAAATATTGGATCTCCTGGGATCAATCGGAATATT
AGCCTTAATTGTTGATCTTAAAGCCTTTGTATCTAACTAACGCCATT
GATCTAATCACAATTATAAAATCTGCATATTCTGTATAAGTCTGCATCAT
TTGACGTAACTCTTAAGTCTTGGCTTAAGTGCACACTATAAGGAAGTATT
TATTAGAGACACAAATATTCAAGTCGCCTCATTGAACAAATCGCGAAA
ATTGGCTAGCTGCCAAACTTCTGTAACCAAGGACAATGGTTATTAAA
CCATTAAAAACTTAGACCCACTAGCTCTAGATCCCCCTCAAAAGATTAAA
AAAAAAACACGATACCCATTCTACTGAACCTCGTTTGCTGTCGTTT
TTCAACTCGAACGGAAATGAGCTGACAGCGCACCGCACACGTCATTGCAGA
AAAACATCGGATAAAACAGGAGGAAAAGTTGTGCAAGGTGGAAAACGTGTT
TACCAACTATTGTTAGAGGCCTAACAGAATTACGCAGCTTCGGTTAGTTA
GCAAGGGGTACCGGGAGCGTTACGTTGCATTGCGTATTCCGCTAAATGT

FIGURE 17 (Continued)

CATCGGAAAAGGCAAACGGCGAAATGCAGAACGAAAGTTTTGATTGCCCG
TGTAAATCGATATCGATGCACAAACTATTGCATTGCAACCCTGCAAGAATA
TGCAAGAAGTTGGGGCGGCCGCGCAGGGGGTGAAGTTGAGTGCCTAAG
TTGGCTAAAGCGGAAACAGGAAATGAGAAAATTTCAGAGAACAAACCCGA
ACTGGAAATGCAACTAACTGGGCACATGCACTTGCAGAACATTCATTGGATAGC
GTTAAGAAATTATTAAATTGTAACTAACATTAAATCGTATTCAAAAGCA
ATTAAATCCAATCCAATTCTTATATAAAATCCTTACAAGATTATTCTATTAA
CTGTAAATCTAAGCAAAAACCCCTTGCAAAATATTGCCTGCACAGCACA
GATCAGTGAATAATCAAATGAAGTCTTGCAGAACAAAAACCCCAATTG
CGTGTGGAACGTCCCCAATGCTTTGCTCGGTTCTGACCTGGCCGTGGT
CAGCCCTGTAGAGGATGTCGAAGTCCTGCAAGCAGAGCAACTGCAGCGAT
TGACGCCCGCTGGATGGCGCCAAGCCGCGTAATATGCGCACCTGTTCCAC
ATTGCAGACGAGCGGTACGATGTCAAGGCCTTAGTTGGTATAAACCGTA
TGCAGACCGCCGACCAAGTGCCTCAGGAAGAGCTCGAAGGCCCTGCCAGGC
AGAGCATCGTTCTGCTAATTATGAATGCGCGACCTCTGACCCCGGTAC
TCCACCAGCTGCACTCATTGGCACTGGGATCCGAGGTGGAGATCGCGCG
GTGAGTTGTACGACCTGGCGGCACATGGTATGGCGGCCATCAGTTC
CAAGGGGGAGGCATGGTGCATCATCTGCAGGGAGTTGAGGAGCCCCAGCGA
ATGGGGCGGCAGTCCATGGGCATTCTGGCGGTAGGCCGTCGGCAGTCCG
TTGCCCGATGGCATCCATGTGGCGGGGGCTGAGCTGATGGTGTGCTGCTG
CTGCTGTTGCTGCTGTTGCTGCTGCTGCTGCATCTGCTGCATCATGGA
GTGGTTGAGGGAGGCTACCGGACTGACGGCACTGGGATGGCGACTTGGTGA
CTGGCCGGTGAGCAACTGGATCCTCGTCCTGTGCGAGCTCCGTCCATTGG
ACGATCCTCGTTGGCTCCACCGCAACTGTTGCTACCGTCGCGTCCATTGTGCT
GCTGCTGTTGCTGCTGGGCTGCTGCGGCTGCCGCTGCGGCTGCCTGCTGC
TGCTGCTGGTATGCATCACATGGCGGTGGTTCATATTGCCCGGGCTCT
TTCAATACCACTATTAAATTGTTATTATTGCGCCCGTTATTGTTATT
TTCACTCTGTTCACTGTACAGAACATCCATACTCATGGCCGACACTTTG
TTTATTACTTTAATCGATTGTTAATTGACGTTTTCTATCGTACAAA
AATTGACACAAAGTAAGGGAGAAATAGAAAATAGATGGTGGAGAGGAAGAT
AAATAATTAAATGAACCTTAATTCAATTAAATTATTATTAGGCTCTATATG
CAAATTCTAAAGTGAGCGTGTCTGTATATTCTATCCGCTTATTATTGGCTTA
CATTAAATACTCTGTAAGTTATAACATCAAATTAAATGCAGACCTTC
AAAAAATTACAAACGATTAGGATTGTATTAGGCTAGCTATGCTCCTATT
TATTTAAACTCTATTGAGCCAGTTAGTTAGTTATGGTAGCTACAAGTT
TATATTGCTAAATATTGTTGAAATTAAATATCCTAACAAACATTACTTACA
AAGAAATATAGAGAACTAACAGAAAATAGAAAAGTTCTTCAAGACATTAA
AAGTCGATTATCTCTAACATCCCCCATAAATAATCCTTATCAACAGAACT
ATTGCTTGCAAACCTTGCTTAATTAAAGTTGGAAAAACAAGGCAATGAA
GCTAATTGGATCCTTACTGCCAATTGCAAAATATACCTATTGTCAGCTT
ATTGAATAATTGATAGAACATAGATTACCTTAAGGAGGTCTAAAGT
AATTATAAACTCAACATCACTGACACAAGACACTCGCGCACTTGCTTTG
ATTTCGCTGTGAAATATACTCTGAATATTCAAGTTATTATTCCGATTGC
CCGCTGTGTTAATCGAGTCGAATAACCGTTTCGTACTGGAATTGGAAA
CCGGAGCTGTGTCGGTTTCGAGTACCGTACCGACGGATTGTCACTCAGAGAT
TGAGAGATGGCAGCTACTCCGCTGCGACGGCGACGGTGGCGTCGCTGCCTCT

FIGURE 17 (Continued)

GCTTCTCGCCTCGACTGGTCCTCTCCCCCTCTGTTGGAGAAATCAAC
GAAACGAATTGCATTGAAATGGGAATCGACTGAGAGCGAGACGGCGAGG
CGACGACTGCGAGTGAGCGAGTGAGCGGGCGTAACGAGTGCTATTTTTA
GCCCACCCACACACACGTACGTACGTACACACGAAGCGCTACCGTTAT
GTACTGAGAGAAATGCGCGCAGAAAAGTTTATTGCATTACCTCTTGCGA
ATGACAAATTGTAATGAAAGGCAGTTCAATTGATTCTTCGGATTTTC
GTGGCAGCGACGCCGGCAGCGCGGTGGCGAGACGAGTGCTGTATGTG
TGTGTCTGTGCCTGTGAGAGCGAGCTGGTATCTGTATCTGCATTGTGCAA
AACCAGAATACGAATACGAGTACGAATACGAATGTCGTGCTGCCGTCCACGT
CTCGCATTACACCAATACCAAGGCCAAAAGGGGAGTGGTATGTGCATTGAT
CGGTGTGTTGCATCTGTATATTCTGTGCAACCCGAAAATACAATGA
CAAACGTAATCGCTCTCTCTGCATTGTTTATTATTTCATTCAATTG
TTGTGCGTGTGTTCTCAAATCCTCCCGCTCTCTCTTGGAAAAAAA
ACGTTTTCAATTCAATTCAATTGTTCTCGTCACTGAGCCCTCTCACACC
ATCTCGCCATCTCTGCGACGCTCAGGTGGGCTGCAACCAATAAACACGAG
CGAGCGAGAAAGCAGCATATTGCATAGCCAGTCGTACATGTTGCCTCTC
GCTCGCCCCATGGCGACGCCATTATAAACAAATGACAATTGTTGGCAT
TTTGTGTTGCAAAGTAAATTATAATAATGCATTGCCAGAGAAGAAAAGTAA
AAAAAAATAGCTTACTTCGAGTTGCGCAGCTGTGTTGACAAAAAGCATT
TAATTCAATTAAAGTAAATGACAAACTTCAACGAATTATAACTTTGGGG
CAGTGTGCTATCTTTCCGTCCTAAAGCTTGATTTTGTCAACCGTTT
CCGTTCCCATTGTTCCATTGAGTCCGTTATTGTTGATTCTTTGTGTT
GCTGATTGGAGGAGAGCAGCACTATGGCAGGGCATCTTCTCCACTTACA
CATATTGCGATAATGGGTTTTTCGCTGAGGGCGTTGTTTCGGGTT
CTTATAAAATAGCATTGCTTAAATTCTGGCATCGCACCTTGCCACCTCTAT
ATGTTTATGTACAATGTATCTGAGAGCTCGTCATTTCATTATTGTCTTC
GTTTCGCTCTGCGATTCTCTCCATAACGATTGCCATTCCGTCGCCGAACC
AATCGCATTCCGTTCTCCATTGAGAGTCCATGTACATATTCTTCTATA
TGGGAATGGAATACGTCTTATGTATTGTTGCACATGACGTATGAATT
TCTTGTGTTGTTGGGCTTTCTTGTGGATTCCCTACCCACTGTCTT
TTAGGTGACAGCAACCATTAAATTAAATTGATTGCAAATGTGGATTCCAA
CAGCTTAAAGAAAATTTGTTGAGAGTCCATGTACATATTCTTCTATA
TTATTGTAATGAAATTAAATTGTTACATCGGTTGTTGATTAAACAAATAA
TTATAAGATTATATTAGATATGAAATTAAATTGTAACGCTACTTTCTA
AACTGTGACATTAGGCTATTCTTACCATGTCATGAAATTCTTCTATA
TTTCATTAAATTGACATATACATGAATCGCTGGCTTAAATTGAATAAGT
ACATTAAATTACCAAAATGACATGCAGAATTAAAAGTATTCAATCAAAC
AAATTGTTTCCCCCATAAATGGACAACAAAAAGGTACTGCCTCTATCATC
CAAGTGTCAAATATGTCAAGCAACCAACTATCGTCAGTAAGAAATGAGTT
CTACAACATGCAACTTTCATGGTGTGCAACTGTGGGGGGAGTTGATT
TTTCGCAACAAACAGCTCGTTGAACCTGGTTTCTCTTAAATAATGCA
ACTGATCTAACTATTAAAGTAAAATTGTATTCTTATTAAACCACAAGCAAGCGC
AAAGATGAGTTATTCTAAAAAGGAGGGTGATTAAATTCTATTAGTT
GGATTACAAATTGGACTAGGAGTCAATTGAAAGTCGTTATATCAATAA
CTTCTGGACTTGAAGCGACAGTTACTGTTCCATAACTTCGATTATCAGCTT
TGCCTCACCATATAGAGTATTCTCTGGATGTGCGAGATTGTATT

FIGURE 17 (Continued)

AAACGACGACTGGATGGCAAAAGTCAGTGCCTCGCAGCTATTATGTGGAT
TATCTGCCTCTGCTGGTCCCCCTGTGGCGGCCAGTCCAATACAAGACTTC
TAAATGGCATCCTAAGTCATGTGGACAAGGAAGCCAATCCCTGTGAGAACTA
CTACAACCACGCCCTGCCGCCAGTACAACATGCGTCACATCGACGACACCTTC
TTCGACATTATAACAAATGCTGGATCACCAAGGTTAACCAAGAACCTGGTGAAAC
TAATGGACGAGCTGGAAATGAGTTCTCAATTGCCGGACTTTAATGTATCTAGT
GTAGATGGCAAGGTCCTCGTTACTACCTTAGTTGTCGTGGAGCGCCCGGA
ATATGGATAGTTAACCGAGTATCTGAAAGTGATTCCCCCGCGAAGGACT
CACATGGCCTCAATTCAATTCCGGACGGTAGTTCTTGCCCCCAGGAGAACATTCA
AATGGCTCAAGGCAGTGGCTCATCTGCATCGCTACGGTCTAACTAACGTGTT
TTAACCTGAAGTCGTCAAACCCACGAAATGCCAGCGAGTACATGGTAG
AATTAAATACACCCACTTTGGAGAAGAACATCTCAACTGCCAACAGAGTTTATT
GAAATTCTATCCGTTCTCTATATCATAAAAGGTTCCAGTGAAATCATTAC
TCTGGCGCGAAAAATGCGAACGCTGAATTGTCCTAAACGATGATCAAT
CCGATCGACACACTGAATAATAGATACATTAGTATCCGCGATTTCAGATGG
AAACCGGTACAACACTGGCAGCGTTCTTGAGATTAAATAGGCTCCAGCGCA
GCCCGAGAACTCCAAGTGTGGTGCAGCAATTAGGTACTTACCGCCCTAA
GGAACATAATGGACAAACAGGATGCTCGGCTGGTGGCCAGCTACATAATGACC
CGATTGCAATATTCTATTGGATGAAACCATGGTGGCAGAGAACATCCACGG
AGTGTGTGTACAGGTGCCGCAACATGAATTGTCCTAAACATGCTCTA
TAAGGAACGATTTCGAAGACTCCACTTCAGTGCCTAAATACCTGGAAATTAA
AGGACATTTCGAGAAACTACGCCATCAGTTCTGCTGCAAGTCGATCAAAA
TCATCTAGAGTTGACTGCTTGCAGATGAAATTTGTCCTGAAAGGCAGAGG
CAATTGAGATCAACGTTGTAATCTCCAAAAACCGATGATCTCGCCATTTC
ATCGGCCAGTACTACCAAGACTTGCAAGTTCCACTGGCAGCTGGATTACC
ATCAGGAGCACCTCAAGGTGCTGCAGTTGCACCCAAAAGATGTTGGCCA
ATCCAGCAAAGGGACTCAGAGGAGCAGAATATTGACTACAGGAGCCAA
GCGCGCCATTGCCTCCACCTCGTACTATGTGATGCCAGCTGGATTATT
GTCCCCCTGGCTACTGCAAGAGCCATTCTCAGCTGGAAAGCGAAGATG
TCTTCAAATACAGCCTGATGGATATTATGGCACATCACTGATAAGCGCC
TTGCCACCGAGGGCATTACAATTGGCAGCGATGGAAACGATCAATCATTAA
GATCGCATCGTTGCAAGAACGAGTCAGTTGCTTCAGCGAATTCAAGAGAA
CATCGATGAAAGCATGGCGATTGCTGGTTAGAACTGGCTATTACTT
ATGCTAAGATGGCCAAGAACGAAACCGTTGGATTTCACCCATTGCCACC
GGAGCAGATATTCTCCTAAATGTTGCCAGTTCTCTGCCAATAGCGATA
TGTGGTTCAAGTACAAGGAAGATCAAGTGCCTTACAGCGAGCTATTGAAGG
GTTTGAGCCATTGACAAGGCTTGGGTGCTACCGCAATAAGCCTAACGAC
GAGAAGTGTGTTATAGTGAATAACCTGTACATATGCTTAGAAATACATATT
TTTGATAACAATAATACAAGACAAATCGTGTAAATTATAAAAGTGTACAAAT
CACATCCATTCTGTTCTTAAAATTAGTTAAACTAACAAATAGTCATAGG
CTAAGATAGTTAAATGATCATCATTGCAATAAACAAACGTTCAAGATTGACTCT
TCAATGTCATGCACCTGCAAGATTACCAATTATATAAAATTAAAAACACAC
AAAGTTACGTGTGTTACTTACATGCATTACATTGGGCCCTGGCCATCCACT
TAATATACTGAGATGTAGCGGTCTTGATTGCGGGATCTCTTATGGATTAA
GAACATTGTTAACTTGCTGACAAAGTAAATTCAACTTTAACGACTTGTGGT
GTGTGCGGCCCGATGAAATGTCTAAAATACAAATTAAATACAATTCAAATA

FIGURE 17 (Continued)

TAATTCAAGACGTAAAAGTTAAAGTAAAATATTTACCTTTAGTGTT
ATTTATACTGATGAGCCTGAAAACACAGTTGAATATCAAACGGATTTGTT
ACCAACAGATTCCAACAGATTCTCAACTTCGTTTTGATTGCCTATTCACT
CGAAGATCTATTCCAGTACTATGATCCTCCATAGTAGAGTCAGCTCAGGATC
TTGTGATAATCCGCAAGCAATTGACAAAGAATTGTCGGCCAGAACAAAAT
TTATTAAATCATTGTAGTCATTCTCAGGATCTCTCTTAACGGCAATCCGTAA
TAACGTATTCATTATCTCCAAAATACAGTCGAATTCAAGATTAAATTGCCG
TTTCCGTCCTTTTATAAATATACATACAAATATACTAAGCAATAGACTGAA
ATGAATTCTAGAATTGAGGAAACTAATTATGTACCTTATGAATAACTTTCC
TTACTGTACTAACAAACTAATTAAACAGATTTCATGCCGAATGATTA
CAATCTTATTGGATGATTGATAGAGCTTAGGAATAATGGTTTAATTGG
ATTAAAGAGTTGCGATTAAGAAACGAAGATATTATCTAGTTTGAGAAC
CAGGGTACTTAAATTTCGACCGCGAACGTCAAAACAAGAAGAAGTTCA
TCAACACTGAATTCCGCTTGGTAATCAGCTGATAAGCGTGCTCACGATAGCC
GAGTTCACATCCAACAGATGTTCCCTAGCAGGGTTCAGACCCAAATGATG
ATTATCTTATTGATTAAGCTCCAACACGCATTGCTTGATAATTCAAGGTA
TTATTAGGCTGCTTAATATACAATCCACTTATATTGTTGTGCCATGAGGAAC
ATCGACACGTGAGGATAAAAATATTATTCGATATATTACTCTTGAG
CCTTTGCACACCCCTAGTTGTGTTCCA

FIGURE 18

dMLF 5' region, 8374 base pairs

GCCTGATTGTTTCCACTTGCAGCAGAGGAGCCGGAAAGGAGCGGTAGAGG
CGCACCCAGTGTATCCGGCAAAGGCAAGTCACCCCCAGGTGCGTTCCATGCC
AGCTCTCCGCTGCCTCAGCGATCCGCTACGCCGACCGGCTGATGAGCCAAC
GTGTCCGTGAGGCAGCCGAGCGTCTGCCAACAGCACACGGTGGCCAGTGC
TCAGCGGCATTGGCAATGGAGAGGCACTGGACTGGCAATGGAAATGGC
AATAGCAATAGTAATGGCAATGGAATGGAAACACCGCGGAGACGAATCGC
GAATCACGCGCGACGTCTCATCAACCGATTCAATAGCGAAACGCAGCATA
TCACGTCTAGTTAACGTTAACGACTGGCACTGGGACTGGCAATGGAAATGGC
TAAAGCAGATATAGCATTGTTAAGTAAAAAAATATATATATACCCAGAG
AAACTTACGAAACACTCGAATATGAATGCGACTGCGGATCAGCATCCCACC
CACCCACACACACACGTCTACCCACTCACAGTAGGATATATGTATGTATGTCT
GCATTCAAGCGGATGCACTCCCTCCGTTAGAGGGAACTGTACTTAGGCTAG
AGGAAGCTAAGTGTAAATTATTGTATCGATTATACATATTACCATAC
TAATTAAAGTTAATGTAACGAAAACGCAGGATCAGTAATCTTATTAGTTCA
ATGGTAATCAATGTGCGATTAGCGGATGATCGCCTGAGTCGCACCCA
CAGTCCGCCGGAGGCTCTAGCGTAATCCGGAAGGTGGCCCAATGGTGTC
TTTCCGGTTACAGGAAGCAGCTGGTAGCTACGCAGCAGGCGGACACTATGG
TCTTGATCTCCATGATGGCGAATCGATTGCCAATGCAATATCTGGTCCAGCG
CTGAAGGGAAAAAGCGTAGGGATGACGGTTCTCGGAGTTCTCGGGCGAAA
ATCGCTCCGGCTGGAACTTTCCGGATCGGGATAATGTGGCAAGACGATG
GGTGGCATAGGGCAAATGAAAACGTTGCTGCCGGGCAATGTGTGCTT
GCCAGGCACCTCTCGCCCAGTTACGAGCAATAAGCGGGACACTGGGAT
ACAGACGCAGTGCCTCCTGATGCACATCTCATGTAACGCATCTCGTGCAGA
TCCGTCATCGTGGGAGCTCTATTACTGTCTCGAATATGGTCGCCAGCTCCAG
GACACAGCGATCTGGCACTCGGATTCTGTGTCAGCAGAAAGAGAGTGAAA
GCCACGGCGGCACCCACCGAATCCTGGCCAGCCAGCATAAAGGTACAGGCCT
CGTTGACGATATCCTCCTCGGTGAAGTCCCATTGCTCTCGGAGATCTGATC
ATGTGGTCGAGCAGACACTTCGCTCGTATTGCCATTATTATTGTTCTGGAT
TTGGCAGCTCTGGATCATTGCGTGTGAAGTCATTGAGGCGCTCTTCT
GGTTAAGCTCATCGTGGCCATCTGGTCCAGTGGTAGATCCGTCCAGCAGC
AGCAGGGTTGCGTAAACCGCGGGCATCATGATCTGCCCTGGCGGAACG
GCGAGTCCTCCATCATGCCACATCCTGACCTCTTCTGATCGGCACACCC
AAAACGGCTCTGCAAGTCGTCAGGGATTAAGTGAGAAATTATAGCTTGCT
AATCCCCTAGAGACTCACCATTAGTATGTCAGTACACAGTTGTTACGTAC
TTGGCAATATTATCTCGTCCCACGGCTCGGCATCCAGATTCTCGTACAA
CGATTGCGAGGCATCCACAAAGGTGCGATGAACTCTCCAGCAGATTGTGA
TGAAACGCTGGCTGGATGAGCCGTCGATGATTGCTCCACTTGGAAACCACTGC
TGGTTATCAGCCCACCCAGGAAATTGTGCATCAGTCGGTAGAAGAAGAC
CTTGGTGGTGTGCTCTCGAGGAGAGTATCACCTGAGATCCTCCGGCTCCA
GGACAGCAAAGAAGGGAAAGAGCAGCACCCAGATCCGCACCAGAGATCCAT
ATAGATCGAAGGCCTGCCGGCACATCTGCGCATCACTGTGAAATGGGATT
AATTAACTAAAGGTATCTTCACGAAAAGGTTCTCAAGGATCTTACAG

FIGURE 18 (Continued)

TCCTTATCCGTGACCAGCATGCAGTTGCCAGAAATGGCAGCGATGGCGGAC
CCGTGAGTCTCAGCGAGAGGAACCGATCTCAAGTACGTGTCAGGGTGCG
GTAGAATGTGTAGATGCTCAGGCTGATCACCAAGGAGGATCAGAACGGCAT
AGCTCCAAATTGGTGGTGCCTCCAGCTGTGGGGGCAAAGCAAACGTAAAT
GCATTGGGTCAAGTCGCGTGGATAATTGCCGCTTAGGTCAATATTGGTTG
CTATCGAGAACGCCAGCTCTGAACGCACCTCATCAGCTACGCACTGCGCTC
ACTGGAGTCTAATTAACAGAGGAATCTGGAGCACTTAGGCATTCGAACCTG
GATGCGAGCACTGCCCTGCCGCGTCGCAAGTTTCGGCAAACACACGTT
ATCGTAATCGCAACGAAAGTATAAGTTATGTATCTAACACTGCGGTGTGAATTG
CTTGGGCCAACATGGCGTATGGCGATGCTATAAGTACGTGTGTGTATCC
ATAAGATCGATTAAAGCACCACACCGTTATGTGTACGTGTTGCTTGCTTG
GTTTTTTCTTATTGGGCCATTGCGTCGATGTTCGTGGTGCAACAG
GTTACACGATGAGCACAAACATGACAAATGATGATGATCACCGGACAAAAA
ATCCAGGGACAGCCTTGTGCCCACACTCCCACACCTGTCGTCGCCAAC
ACCCTTGACCAACTAACCCCCCCCCCCCCAAAAAAAACCCCTTGTGTTG
GTTCTGGACGAGAGTGAGAGCCCCAACACCAATTAGCCAATGCGATTGGTT
CAGGGCCAAGTGAAACCACCGGTTGGTAACTGACTCAGATCTCAATGATTA
ATTATTACGGACAAGGAATCGGCAAACGATCGCAGTTGGTCATCATAAAGT
TTATCCAAAAATCTAGGTGGCATTCCATTAGTGGAACTTCTTACCATCAGT
TCGTAGTAAGCTAAGTAAAGAGTAAAATAATAGGCGCTTTAATCCTCCTCA
GCCACCTCATCCTCGTAGCCCTGGCAGGGCATTCACCTCGGCATTGTGAAA
CAAACTGCATTGCCATCTCCCCTGGGAATCGTTGGTCCGCCCTCAGGT
ACTCGTACTGGCAACGGCTCCCGCTCCACGTGCTGTGTCGGTGAAGGGG
TTTGGCGCGCACAGGACGATGGCCGGTAGGGCCAGCAGGAAGGTGACACGC
TTCCACAGACCAGCGGTATTGGCGGGCATATTGCCATAAGCCGGATATCAA
AGATCGGCATGTACTGCACAATAACTCGACTTATTCTGATGCCTGGCTGGAT
GGCTAAGCTAGCTGAATTGAATAACTACGTACTGTAGCGATTGAATCTGAT
CCGTAACACCCACGCCCTGCTGCCAAACTATTGTCGCAATTAGGAACCTCA
AGGGGATCCGAGGCCAGGCCACAAGGTCCAACAAACCCGGTATCTTGTGTTA
ATCAGCCCAATTGACCAAGAAACCGCTGAAGCGTCCAGAAGGCTTGCCT
CCGCTCCAAGCGGCTACTTCTATTTTTCTTCTCATCGAATATTCTTCTT
AGTTGCAATGCTCTTTTTGGCAGGCATATCCATCCCCATCCAGG
CTCGAGGGTGTGAGAACCCGCCGTGTTGCTGCCAATTGGCACCTGGC
CACTAATAGATATACATCATGATTATTCCACTAATTCCATAAGTTATCATA
ATGGTCTCCTAAACGAGAGGCTGCTGTGAGGCCTAAAGACCGCCAAAA
TCTAACGATCCATTGAGATTGCGGTTAAAATGATTCAAATGCAAGCGAAGT
TACTAAAATTGTGAGAGTATATCTAGTTGAAAACCTGAACCTGAAAATGTG
GTTTCATAAAATTATCCAAATTGATGGGTGTGAATTAAAATTAAATTAAAAA
CTTGCACCTGAATACTCAAAATTGCTCAATTGATAGATAATGAT
GATTAAATAATAAGTTATAAACCTAGATCATTCACTTCTTACTGTTATG
AATTAGGTTATATATCCTCACTCTTAAAGTAATGAAATTGTTATC
CTTAGGAAATACACCTTATTAAAATTATTAAATTCTTAAATTCTATTAAAATTCTT
TTAAAAAACAGAAACGTAATAGCCACCTTACATTACTAAACGTTT
CCTTTCTTTAAACTTAGCTGTGAGTAATCCTTTTATTCTACAAACGAATT
GCGTTAAATATTATTTCTCACTCACCACCTTCCACAAACATT
AGTCACGTATTGTATTCCCTGATAGTCATATATTGTTTATCTTAA

FIGURE 18 (Continued)

ATAGCTCACACAAAAGTCCTGCCACAAGCACTGTCAAATCCACACATAC
ACCAAGTTAGTTAGCTCCACTTCGATTGGGATATATCCGTATTGTGATCTTA
TTGGCCAGAGTCACATCCGGCAGTGTAGCTACGAGTGCAGGACCTCCGC
CGGTTAGCGTCTATTATAACCGATTGGCCCGATCAAGCTCGGCTTGAACGC
CGCCGAAAATGATGTACGTGCTAGCTAAGTCGTTGGAGTCCCCGGATACCCG
AATCCCCGTATCACCAGAACATACCAAAATCGCCAGTCGCCCGTATCCGCTAG
ATGCCCGAGTGTATCGAGTATAGGTAGTAATTGCCAACTAGTCGGCACTCGA
AGTCTAAGTAGCTAGAAGTGGATATGGTGCTGGATGCTGGATGCCCTGCC
AAGTGGCATGGCAATCAATTATCCGTTGGTGCTTGTGATGTATCCCTCCT
CCGCCACCGCCCACGCTATCCACCTCCTCCATGGAATGGCAGACCCTTGGGT
TGCCAGTGGCACTCAACGATCTGAGCGTGGAACAGAGGGGAAGTCAGCT
AGAAATCTCAGACCGTGGCAGTGGATCGAACCTTGAGCGGATATTCAAAT
GGCGCAGACGGACCTCTCACGTTGATTACATCAATCATTTATGTTACA
GTGTAGTCGTCGGATCTTGACATTCACATACTGTGCTTTAACGTACATCAT
AAATTCTACAAAATATATTGGGATTATTCCCGAAATTAAACCTTGCT
AACTTCAATTGTTACTGAACACAAACGAATTACGTTCTTATTCAATTCAATT
ATCATTCACTGTTACTGAGATGTGTTAGTCAGTTAACGATCGTTGAGTTAG
GTTAGAAATCTTGGAAATTCAATAGCGCATTGGTACTGATTAAGAGTTATT
ATCAGTAAGAATATTATTAGTAATTATTATGCCAATCAGACCGATTAGAC
TACCACTTCTGTACTTTGCTGCGAGTCTCGTGCACCACCGATTAATATGGT
AAATAAAATCTCAGCCTGCTTCCAACACCACTATCTGAAGACACGATTCCA
TGGAGCACATGGAGATTGAGATTACAGCCATCGACTAGACGCCCTCGTCATT
CGGGACGATTAAGTCAGTGGCAAATGAAATAGAGTCGATCGATGACTCAA
CGGATCGCTTGCTGATAATCCCCATTGTTCTCCTTAGCTGGCCAGTTGA
CTTTTGGTCAGTTGACTTTCTGGCCAGTTGGCTTCTGGCCATTGGGTCTT
AGAAGACCACCGCCCAGTTATAATGGATATAAAACGAATTGAGCTGCAAGT
CGTATAAAACTTACGGATATCATAGCAGAAGTTATGAAAATCCAAATACCA
ATCATGGATGATCGCTAAATTGCCATTGGTGATAAGTGATAAGCTGGCTA
CTCCAGCCCTATATAAGAGACCTAAATCGAACCCACACTTAAGTTAACCATG
TCGCTACGTTGGTTGTTCTTGGCTGCACCTGGTGTGTAATTCTCACG
GATTCCGCCTCCATAAGCACCCACATTGTTGGTGGCGATCAGGCGGACATCG
CTGACTTTCCGTACCAAGGTGTCCCGTGCCTGGAGACCTACATGCTGCTCCAC
ATCTCGGGTGGTAGCATCTATGCACCAACGGGTGTCATCACCGCCGCCCCACTG
CATCAAGGGACGCTATGCCCGTACATCCGGATCGTGGCTGGTCAGAAACTCG
ATTGCCGATCTGGAGGGAGCAGGGTGTAAAGGTAGCAGCAAACGATCCCCCATG
CCGGCTACAATAAGAAAACGTATGTGAATGATATCGGTTGATCATCACTCG
CGAGCCATTGGAGTACTCAGCCCTGGTGCAACCCATTGCTGTGGCCCTGGAG
GCACCGCCGTCGGGTGCCAGGCCGTTGTAAGTGGTGGGGCAAGCAGGGCTG
AAGATGATGAAGCTCTGCCGCCATGCTGCGCGCCGTTGAGCTGCAAGATCAT
CGAGAAGAGCACCTCGGGCCAGTATCTGACCAAGGACTACACGGTGACC
GATGAGATGCTCTCGGCCGGCTATCTGGAGGGCGGCAAGGACACCTGCAACG
GCGATTCCGGTGGACCCCTGGCCGTGGACGGAGTCCTGGTGGGTGTGGTGTGTC
CTGGGGCGTGGGTGCGGCAGGGAAGGATTCCCGGGTGTACACCAGCGTC
AATTCCCATATCGATTGGATCGAAGAGCAGGCCGAGGCGTATCTCTAAAAAT
GTGGATAGCTTCACAAGCACAACGCGAACAAATAATCGAACAAATTATTAT
TTTACCAATAATAATGAAATGAGCATTAGAAAACATGGTTATAAT

FIGURE 18 (Continued)

ATATTACAAATTAATACGGTHTTAACTCTCATTCAACTGGTTTCCTA
ATCAAAAACCTTTATCTGACCATTACATTGGAATCTATAAGCCATTCTCG
ACGATTATATAAAAATAAAATTATTACCCAATTGGCATAGGTGAAGGCAT
TTATCTTGAGGAAGGGAAAAAGTACAATGTAACTAACCATAAATTTATACT
TTACAAAATCGTTGATTGCATCATTAGAATAACTCAATGCAGAAATTAAA
ATTATAAAATATGTAAATGTGGCTTGAAGTATCATTATTATTGTGACA
TTTATATTGACTTGATGCAATCAAATAATATCCACAATTAGAAATTAC
GTTTGCAGATAGTTAACGTATTGAGTAAGATTACATTGTTAAATCTTAA
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ACCGTTCACAAATGGAGCTCCAGTAACTCGCACATCAGTCAAGTATCACTAA
GTTACTCATTTCGTTGCAG

Attorney's Docket No.: 066 X-686001
 Client's Ref. No.: CIT-3056

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled:

AN ANIMAL MODEL OF POLYGLUTAMINE TOXICITY, METHODS OF USE, AND MODULATORS OF POLYGLUTAMINE TOXICITY,

the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status
60/148,934	August 12, 1999	Converted
60/148,933	August 12, 1999	Converted
60/177,047	January 18, 2000	Converted
60/205,720	May 19, 2000	Converted

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Serial No.	Filing Date	Status

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed

Attorney's Docket No.: 06618-686001
Client's Ref. No.: CIT-3056

Combined Declaration and Power of Attorney
Page 2 of 2 Pages

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Scott C. Harris, Reg. No. 32,030; Robert M. Bedgood, Reg. No. 43,488; Gregory P. Einhorn, Reg. No. 38,440; Michael P. Reed, Reg. No. 45,647; Kenyon S. Jenckes, Reg. No. 41,873; Bing Ai, Reg. No. 43,312; William J. Egan, III, Reg. No. 28,411; David L. Feigenbaum, Reg. No. 30,378; Hans R. Troesch, Reg. No. 36,950; and John R. Wetherell, Jr., Reg. No. 31,678 of Fish & Richardson P.C.

Direct all correspondence and telephone calls to SCOTT C. HARRIS, PTO Customer No. 20985 (Phone No.: 858-678-5070).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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